Tryptophan-metabolizing gut microbes regulate adult neurogenesis via the aryl hydrocarbon receptor

George Zhang Wei\textsuperscript{a,b,1,} Katherine A. Martin\textsuperscript{a,b,1,} Peter Yuli Xing\textsuperscript{c,d,} Ruchi Agrawal\textsuperscript{1,} Luke Whiley\textsuperscript{a,1,} Thomas K. Wood\textsuperscript{g,} Sophia Hejndorfh\textsuperscript{b,} Yong Zhi Ng\textsuperscript{c,} Jeremy Zhi Yan Low\textsuperscript{1,} Janet Rossant\textsuperscript{c,} Robert Nechanitzky\textsuperscript{d,} Elaine Holmes\textsuperscript{c,} Jeremy K. Nicholson\textsuperscript{b,m,} Eng-King Tan\textsuperscript{b,} Paul M. Matthews\textsuperscript{b,n,o,p,2,} and Sven Pettersson\textsuperscript{a,b,h,q,2,}

\textsuperscript{a}Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 639791; \textsuperscript{b}National Neuroscience Institute, Singapore 168857; \textsuperscript{c}The Singapore Centre for Environmental Life Sciences Engineering, School of Biological Sciences, Nanyang Technological University, Singapore 637551; \textsuperscript{d}Interdisciplinary Graduate School, Nanyang Technological University, Singapore 637335; \textsuperscript{e}Australian National Phenome Centre, Health Futures Institute, Murdoch University, Perth WA 6150, Australia; \textsuperscript{f}Perron Institute for Neurological and Translational Science, Nledlands WA 6009, Australia; \textsuperscript{g}Department of Chemical Engineering, Pennsylvania State University, University Park, PA 16802; \textsuperscript{h}Department of Neurobiology, Care and Society, Karolinska Institutet, 171 77 Stockholm, Sweden; \textsuperscript{i}The School of Biological Sciences, Nanyang Technological University, Singapore 637551; \textsuperscript{j}Program in Developmental and Stem Cell Biology, Peter Gilgan Centre for Research and Learning, Hospital for Sick Children, Toronto, ON M5G 0A4, Canada; \textsuperscript{k}Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, ON M5G 2C1, Canada; \textsuperscript{l}Section for Nutrition Research, Imperial College London, London SW7 2AZ, United Kingdom; \textsuperscript{m}Institute of Global Health Innovation, Imperial College London, London SW7 2NA, United Kingdom; \textsuperscript{n}UK Dementia Research Institute, Imperial College London, London SW7 2AZ, United Kingdom; \textsuperscript{o}Department of Brain Sciences, Imperial College London, London W12 0NN, United Kingdom; and \textsuperscript{p}Faculty of Medical Sciences, Sunway University, 47500 Kuala Lumpur, Malaysia

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While modulatory effects of gut microbes on neurological phenotypes have been reported, the mechanisms remain largely unknown. Here, we demonstrate that indole, a tryptophan metabolite produced by tryptophanase-expressing gut microbes, elicits neurogenic effects in the adult mouse hippocampus. Neurogenesis is reduced in germ-free (GF) mice and in GF mice monoclonized with a single-gene tnaA knockout (KO) mutant Escherichia coli unable to produce indole. External administration of systemic indole increases adult neurogenesis in the dentate gyrus in these mouse models and in specific pathogen-free (SPF) control mice. Indole-treated mice display elevated synaptic markers postsynaptic density protein 95 and synaptophysin, suggesting synaptic maturation effects in vivo. By contrast, neurogenesis is not induced by indole in aryl hydrocarbon receptor KO (Ahr\textsuperscript{−/−}) mice or in ex vivo neuronspheres derived from them. Neural progenitor cells exposed to indole exit the cell cycle, terminally differentiate, and mature into neurons that display longer and more branched neurites. These effects are not observed with kynurenine, another Ahr ligand. The indole-Ahr-mediated signaling pathway elevated the expression of β\textsuperscript{-}catenin, Neurog2, and VEGF-α genes, thus identifying a molecular pathway connecting gut microbiota composition and their metabolic function to neurogenesis in the adult hippocampus. Our data have implications for the understanding of mechanisms of brain aging and for potential next-generation therapeutic opportunities.

microbiota | tryptophan metabolism | indole | aryl hydrocarbon receptor | neurogenesis

The emergence of nerve cells was a major evolutionary transition required for the formation of multicellular life and, remarkably, predates the emergence of the mesoderm. That is, nerve cells appeared before the mesoderm layer, which, among many different cell types, control blood cells and adaptive immunity (1). Neural stem and intermediate progenitor cells reside in specialized niches of the adult mammalian brain and give rise to new neurons throughout life. In contrast to neurogenesis in early life that requires appropriate stimulation at “critical periods” in development to establish functional neuronal circuits (2,3), adult hippocampal neurogenesis (AHN) requires continuous stimulation throughout life (4). The current view holds that AHN functionally contributes to learning and memory (5–7) as well as regulating the hypothalamic–pituitary–adrenal (HPA) axis in response to stress (8). Adult neural stem cells (NSCs) largely reside in a mitotically dormant, quiescent state (9) but can be activated and respond to interventions, including physical exercise and diet (10–14). Therefore, it has been suggested that new neurons are generated “on demand” in response to environmental stimuli or stressors (10). This raises the interesting prospect that AHN may have conferred evolutionary advantages to mammals, for example, in mediating a metabolic stress trigger of food-seeking behavior for survival. Mechanisms by which environmental signals regulate adult neurogenesis are incompletely understood, although circulating hormones and growth factors including adiponectin brain-derived neurotrophic factor and vascular endothelial growth factor (VEGF) have been implicated (14–22).

Significance

While the effects of gut microbes on brain development and function have been described, the mechanisms remain largely unknown. Here, we report that tryptophan-metabolizing gut microbes secrete indoles that regulate neurogenesis in the adult hippocampus. This stimulatory effect on adult neurogenesis is mediated by the metabolic- and immune-linked aryl hydrocarbon receptor (Ahr). Another Ahr ligand, the tryptophan metabolite kynurenine, failed to induce neurogenesis, suggesting ligand specificity of Ahr-mediated regulation of adult neurogenesis. The indole-Ahr signaling pathway elevates transcription factors and signaling proteins that promote adult neurogenesis, as well as key markers of synaptic maturation. Our data demonstrate a symbiotic gut–brain coregulatory axis that connects the metabolic status of gut microbes to the control of neurogenesis in the adult hippocampus.

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G.Z.W. and K.A.M contributed equally to this work.

To whom correspondence may be addressed. Email: sven.pettersson@ki.se.

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Animal evolution and cell fate specification, including cells of the nervous system, are influenced by the presence of microbes. Following initial observations that gut microbes affect the postnatal development of the HPA axis stress response (23) and alter host brain development and anxiety behaviors of germ-free (GF) mice devoid of microbes (24, 25), gut microbes have rapidly attracted attention for their roles in gut-to-brain communication [reviewed by Kunda, Blacher, Elimam, and Pettersson (26)]. Indeed, several hippocampal-dependent behaviors are included in the growing repertoire of functions linked to microbes, including fear extinction and anxiety-like responses (23, 24, 27–29).

Gut microbes are an evolving, prokaryotic component of the metaorganismal self. They secrete myriad metabolites of which several are known to regulate cell function and the integrity of permeable barriers, including the blood–brain barrier (BBB) (30, 31). Importantly, the microbial metabolic output is dynamic, allowing microbes to respond to environmental cues, including nutritional fluctuation. Indoles are microbial metabolites of dietary tryptophan that are produced in response to conditions of low glucose availability and act to adaptively inhibit microbial replication (32–34). Indeed, diet appears to be a major regulator of gut microbiota composition and function (35, 36). An increasing range of observations suggest that changes in the gut microbiota may influence the brain. GF rodents exhibit elevated tryptophan levels and reduced indole derivatives in serum (37). In murine models of multiple sclerosis and of encephalomyelitis mice, the microbial metabolic output is dynamic, allowing microbes to respond to environmental cues, including nutritional fluctuation. Indoles are microbial metabolites of dietary tryptophan that are produced in response to conditions of low glucose availability and act to adaptively inhibit microbial replication (32–34). Indeed, diet appears to be a major regulator of gut microbiota composition and function (35, 36). An increasing number of observations suggest that changes in the gut microbiota may influence the brain. GF rodents exhibit elevated tryptophan levels and reduced indole derivatives in serum (37–39). In this context, a correlation between reduced serum indoxyl sulfate (a liver metabolite of indole) in GF or antibiotic-treated mice and impaired fear extinction learning has been reported (37). Translationally, the administration of indole, indole derivatives, or microbial tryptophanase enzyme to antibiotic-treated experimental autoimmune encephalomyelitis mice—a multiple sclerosis model system—reduced CNS inflammation and improved disease scores by the activation of the aryl hydrocarbon receptor (AhR) pathway in astrocytes (40). In humans, while several studies have associated diseases of the CNS with altered tryptophan metabolism (41–44), the clinical potential of indole remains to be elucidated.

The aryl hydrocarbon receptor (AhR) is a ligand-induced transcription factor that, upon binding a cognate ligand, heterodimerizes with the AhR nuclear translocator (ARNT) and translocates to the nucleus to activate downstream target genes. Different ligands are thought to induce particular conformational changes of the AhR:ARNT heterodimer complex, which determines the recruitment of cofactors that establish downstream enhancer and promoter gene activation. Interestingly, the AhR is also a target for tryptophan metabolites, including microbiota-derived indoles and eukaryotic kynurenines [reviewed by Hubbard, Murray, and Perdew (45)]. AhR-dependent pathways also influence host–microbe interactions. For example, in the intestine, AhR modulates the gut microbiota community structure in mice (46, 47), immune activity, and maintenance of the epithelial barrier function (48). Recent data describe how AhR-dependent pathways are important for a wide range of biological functions, including development, metabolic homeostasis, cell growth, and differentiation in multiple tissues, including those of the central nervous system [reviewed by Lee and McPherson (49)].

The spatiotemporal expression of AhR messenger RNA (mRNA) in the developing embryonic brain as well as the juvenile and adult mouse hippocampus suggests a role for this signaling pathway and transcriptional activator in regulating neurogenesis across the lifespan (50). In the adult brain, the AhR is localized in neural progenitor cells (NPCs), granule cells, and astrocytes of the dentate gyrus (DG), where its signaling has been associated with NPC proliferation, fate specification, and dendritic development (51–56). Moreover, deletion or 2,3,7,8-tetrachlorodibenzodioxin (TCDD) activation of the AhR diminishes NPC proliferation, neuronal differentiation, and impairment of contextual fear memory behavior in mice (51). These results suggest a link between tryptophan-metabolizing gut microbes, AhR signaling, and adult neurogenesis. Here, we report the identification of a gut microbiota-derived AhR-mediated signaling pathway that regulates neurogenesis both in the adult mouse hippocampus in vivo and in ex vivo neurosphere cultures.

**Results**

**Gut Microbiota-Derived Indoles Regulate Adult Neurogenesis.** To assess the impact of microbiota on AHN in C57BL/6J mice, we contrasted 3,3-diaminobenzidine (DAB) staining against double-cortin (DCX), which labels proliferating mitotic NPCs committed to a neural lineage (57), between the dentate gyrus of GF and specific pathogen–free (SPF) mice. In line with recent findings in younger mice of the same strain (58), we observed diminished neurogenesis in GF mice compared with age-matched SPF controls (1,020 ± 40 versus 700 ± 30 DCX+ cells for SPF versus GF mice, respectively; P ≤ 0.0001; Fig. 1 A and B).

The BBB is permeable to indole (59, 60), and GF mice display reduced levels of indole derivatives in serum (38). To test whether microbiota-derived indoles modulate neurogenesis, we monocolonized GF mice either with a wild-type Escherichia coliindole+ (WT E. coli) mice or with E. coliindole− containing a mutated, nonfunctional tryptophanase (tra4A) enzyme (MT E. coli) mice (61) (Fig. 1 C). MT E. coli mice had lower serum concentrations of indoles compared with WT E. coli mice (Fig. 1 D and E; P ≤ 0.001). MT E. coli mice also displayed reduced neurogenesis in the DG compared with WT E. coli mice (Fig. 1 F and G; 690 ± 20 versus 510 ± 30 DCX+ cells for WT E. coli versus MT E. coli mice, respectively; P ≤ 0.0016). Providing indole in drinking water (200 μM) to either “indole-deficient” GF or MT E. coli mice for 5 wk increased neurogenesis relative to that of control mice receiving standard drinking water (Fig. 1 H and I; 720 ± 30 versus 1,005 ± 30 DCX+ cells for control versus indole-treated GF mice, respectively; P ≤ 0.0028 and Fig. 1 J and K; 510 versus 630 ± 12 DCX+ cells for control versus indole-treated MT E. coli mouse, respectively; P ≤ 0.0037), underscoring the potential role of indole and its derivatives in the control of neurogenesis.

We next explored whether indole exerted direct neurogenic effects on NPCs using ex vivo neurosphere cultures comprising NPCs and NSCs. NPC proliferation, cell cycle characteristics, and neuronal differentiation were assessed. Indole treatment increased the relative number of progenitors that differentiated into classes III and VI (TuJ1+) neurons compared with vehicle-treated controls (Fig. 2 A and B; 5.6 ± 0.4% versus 8.6 ± 0.3% for control versus indole-treated NPCs, respectively; P ≤ 0.0001). No differences in total cell numbers or programmed cell death reflected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay were observed (SI Appendix, Fig. S1 A and B). To assess whether changes in NPC proliferation contributed to the observed phenotypes, NPCs were labeled with 5-Ethynyl-2′-deoxyuridine (EdU), a widely used marker of proliferation (62), 1 h before treatment with indole and 24 h prior to staining against EdU/Ki67 (Fig. 2E). Neuronal progenitors treated with indole displayed a lower proportion of EdU+/Ki67− labeled cells after 24 h (Fig. 2 C and D; 49 ± 2% versus 36 ± 2% for control versus indole-treated NPCs, respectively; P ≤ 0.0001), but there was an ∼10% increase in EdU+/Ki67+ cells (Fig. 2 E, Middle and Right; 19 ± 1% versus 30 ± 2% for EdU+/Ki67−/EdU+/Ki67+ cells for control versus indole-treated NPCs, respectively; P ≤ 0.0008) compared with vehicle-treated controls. Thus, indole promotes the exit of NPCs from the cell cycle and commitment to neurogenesis. We then tested whether these effects generalized to all AhR ligands. In contrast to the microbiota-derived indole, treatment with kynurenine—the major tryptophan metabolite of eukaryotic cells—did not induce any changes to the extent of neurogenesis (Fig. 2; 1.7 ± 0.2% versus 1.4 ± 0.2% for vehicle- and kynurenine-treated NPCs, respectively; P ≤ 0.64965). Moreover, a slight increase in the proliferation capacity and proportion of NPCs retained in the cell cycle was observed (Fig. 2G; 55 ± 1%
versus $58 \pm 1\%$ for vehicle- versus kynurenine-treated NPCs, respectively; $P \leq 0.1655$ and Fig. 2H; $71 \pm 4\%$ versus $77 \pm 2\%$ for vehicle- versus kynurenine-treated NPCs, respectively; $P \leq 0.4476$). No evidence of impaired kynurenine transport into NPCs was found (SI Appendix, Fig. S1C and D).

Further characterization of ex vivo neurospheres revealed that indole promotes neuronal maturation. Neurons differentiated for 4 d either in medium supplemented with indole (100 μM) or vehicle-only medium were identified by Tuj1 immunostaining, and those in fields of comparable cell density (Fig. 3A) were

Fig. 1. Exposure to indole rescues neurogenesis deficits in “indole-deficient” GF and mutant E. coli monocolonized mice. (A) Representative images of DCX-DAB-stained immature neurons in the DGs of SPF and GF male mice. The black dashed boxes indicate the comparative areas that are magnified to show the notable increase in DCX+ neurons. (B) DCX+ immature neuron populations are significantly reduced in the DGs of GF (n = 7) compared with SPF (n = 13) male mice. (C) Male and female GF mice were inoculated with WT E. coli tnaA or mutated E. coli ΔtnaA and their progeny maintained in a controlled environment until experimental testing. (D) WT and MT E. coli indole production was qualitatively assessed by Kovac’s assay, whereby the presence of indole is indicated by the presence of a pink color change in the alcohol layer of the reaction mixture. (E) Liquid chromatography–mass spectrometry analysis showing concentration of indole acetic acid is significantly reduced in serum of MT E. coli compared with WT E. coli male mice (n = 10/group). (F) Representative images of DCX-DAB–stained immature neurons in the DGs of WT E. coli and MT E. coli mice. The black dashed boxes indicate comparative areas that are magnified for clarity. (G) DCX+ immature neuron populations are significantly reduced in the DGs of MT E. coli male mice (n = 8) compared with WT E. coli male mice (n = 5). (H) Representative images of DCX-DAB–stained immature neurons in the DGs of GF male mice treated with sham or indole-supplemented drinking water (200 μM) for 5 wk. The black dashed boxes indicate comparative areas that are magnified for clarity. (I) DCX+ immature neuron populations are significantly increased in the DGs of male GF mice supplemented with indole (n = 4) compared to vehicle drinking water (n = 9). (J) Representative images of DCX-DAB–stained immature neurons in the DGs of MT E. coli male mice treated with sham or indole-supplemented drinking water (200 μM) for 5 wk. The black dashed boxes indicate comparative areas that are magnified for clarity. (K) DCX+ immature neuron populations are significantly increased in the DGs of MT E. coli male mice supplemented with indole (n = 7) compared with vehicle drinking water (n = 8). In all images, nuclei are stained with DAPI (blue). (Scale bars: 100 μm.) All data are presented as mean ± SEM. Statistical differences were determined using Mann–Whitney U test (B, G, I, and K) and Student’s t test (E). Asterisks indicate a significant difference between groups (****P < 0.0001, **P < 0.01).
analyzed by Sholl (Fig. 3B) and “inside out” (I/O)-labeling schemes (as described in ref. 63) (Fig. 3E, Left). Semiautomated Sholl analysis showed that indole-supplemented neurons displayed ca. 32% more terminal branches (Fig. 3C; 5.6 ± 1 versus 9.4 ± 1 for control versus indole-treated neurons; P ≤ 0.0112) and a greater degree of branching along their length (Fig. 3D); indole-supplemented neurons had a greater number of primary, secondary, and tertiary branch points (Fig. 3E; 3.5 ± 0.2 versus 4.9 ± 0.3 primary branches, P ≤ 0.0003; 3.6 ± 0.3 versus 5.9 ± 0.3 secondary branches, P ≤ 0.0001; and 2.3 ± 0.3 versus 7.3 ± 0.8 tertiary branches, P ≤ 0.0001 for control versus indole-treated neurons, respectively). Moreover, neurons differentiated in indole-supplemented medium had longer neurites (Fig. 3F; 78.0 ± 4 μm versus 94.4 ± 4 μm for control versus indole-treated neurons, respectively; P ≤ 0.0032). Neurons analyzed after a 24-h exposure to indole followed by differentiation for 3 d in vehicle-only medium (Fig. 3G) did not display enhanced maturation (Fig. 3H and I).

**Indole Induces Adult Neurogenesis In Vivo.** We explored the potential for stimulation of neurogenesis by an oral administration of indole. Supplementing the drinking water of WT C57BL/6J mice with indole (200 μM) for 5 wk increased numbers of DCX+ cells in the DG (Fig. 4A and B; 1,051 ± 734 versus 1,370 ± 40 for control versus indole-treated mice, respectively; P ≤ 0.0177). Indole supplementation did not have any effects on water intake or body weight (SI Appendix, Fig. S2A and B). Moreover, we found evidence for the functional integration of neurons generated after indole supplementation through an assessment of synapse expression based on the presence of presynaptic synaptophysin (SYP) and postsynaptic density 95 (PSD-95) in the hippocampus (64). Indole-supplemented mice displayed increased PSD-95 and SYP mRNA in the hippocampus compared with controls (Fig. 4C; 1.3-fold, P ≤ 0.0079 and 1.2-fold, P ≤ 0.0011, respectively). The expression of these proteins was correlated with similar elevations of PSD-95 and SYP protein levels (Fig. 4D; 1.5-fold and 1.3-fold, respectively, P ≤ 0.05). The expression of genes associated with synaptic function/plasticity, including AMPAR GluA1 subunit, vesicular glutamate transporter (vGluT2) and calmodulin kinase II (CaMKII) were increased in the hippocampus of indole-exposed mice (SI Appendix, Fig. S3A–D; 1.7-fold P ≤ 0.07, 1.5-fold P ≤ 0.05,
and 1.5-fold $P \leq 0.05$ compared with control mice). VEGF mRNA and protein were both increased by indole supplementation (Fig. 4F; $P \leq 0.01$). In addition, of the receptor tyrosine kinases through which VEGF signals [VEGFR1, VEGFR2, and neuropilin-1 (NRP1) (21)], VEGFR2 and NRP1 mRNA were up-regulated ($SI$ Appendix, Fig. S3E and G; 1.5-fold, $P \leq 0.05$, $P \leq 0.01$, respectively, compared with control mice). We also found increased expression of the proneural basic helix transcription factor Neurogenin-2 (Neurog2) at both the mRNA and protein level (Fig. 4E; 1.6-fold, $P \leq 0.05$ and 1.8-fold, $P \leq 0.05$, respectively). VEGF and Neurog2 are downstream targets of the Wnt/β-catenin pathway, which promotes different stages of adult neurogenesis [reviewed by Varela-Nallax and Inestrosa (65)]. We therefore probed additional targets in this pathway by RT-PCR and discovered elevated Wnt3a ligand, frizzled receptor (Fzd7), and β-catenin (Ctnnb1) transcripts ($SI$ Appendix, Fig. S3H–J; 1.5-fold, $P \leq 0.01$; 1.4-fold, $P \leq 0.05$; and 1.3-fold, $P \leq 0.01$, respectively).

The AhR Is Pivotal For the Neurogenic Effects of Indole. Indoles activate the AhR signaling pathway. To explore its contribution to indole-dependent neurogenesis, we evaluated the neurogenic potential of indole in AhR-knockout (KO) mice. Indole failed to promote neurogenesis in AhR-KO mice (Fig. 5A and B). Moreover, numbers of DCX$^+$ cells in the DG of indole-treated AhR-KO mice...
were lower than in WT controls. Indole also failed to promote neurogenesis in ex vivo neurospheres cultured from AhR-KO mice (Fig. 5 B–D) or up-regulate Neurog2, VEGF, or β-catenin transcription in the hippocampus of AhR-KO mice (SI Appendix, Fig. S4 A–C).

Discussion

Here, we show that microbiota-derived indole promotes AHN by utilizing a mouse model in which GF mice are mono-colonized with *E. coli* capable or incapable of metabolizing tryptophan. Additional data demonstrate that indole supplementation rescues adult neurogenesis in GF mice. This effect appears to be ligand specific for indole, as a different tryptophan metabolite, kynurenine, failed to induce neurogenesis ex vivo. Similar neurogenic effects were also observed in adult C57BL/6J (male) mice treated with indole. Mechanistically, we observed that indole-induced hippocampal neurogenesis is mediated by the AhR signaling pathway since indole treatment failed to increase neurogenesis effects in either AhR-KO mice or AhR-KO ex vivo neurospheres.

Previous studies aimed at assessing the impact of microbes on hippocampal neurogenesis have revealed somewhat differing results, possibly due to different experimental conditions and timelines for monitoring neurogenesis. For instance, in one study, a different mouse line (Swiss Webster) was used (66), whereas in another, hippocampal neurogenesis was assessed earlier in life (58). A third study reported a decrease in adult neurogenesis of adult male C57BL/6J mice after antibiotic treatment (67), which is consistent with our results. Variation in microbiota dynamics and function (35, 68–71) and differences in the energy composition of the chow fed to mice in these studies may account for some of the discrepancies.

We observed increased adult neurogenesis in three rodent models after treatment with indole, demonstrating that mice respond to environmental indoles beyond critical windows of development. A recent report demonstrating increased AHN in 4-wk-old GF mice after either a transplantation of microbes from 24-mo-old mice or a supplementation of diet with the microbiota-derived short chain fatty acid butyrate are consistent with this (72). There now is a need to assess neurogenesis and the effects of indole supplementation in the fetal brain, where indole metabolites are reduced by antibiotic treatment or GF rearing (73).

Our ex vivo studies display direct, ligand-specific neurogenic effects of indole treatment on NPCs. Consistent with previous reports demonstrating anticancer properties of indole derivatives via cell cycle arrest mechanisms (74), we found that the enhancement of neurogenesis ex vivo was not accounted for by elevated proliferation.
or by a greater survival of neurons but rather by an increased exit of NPC from the cell cycle and differentiation toward a neuronal lineage. Moreover, indole-treated mice display elevated hippocampal Neurogenin 2, a bHLH proneural factor that promotes NPC cell cycle withdrawal and neuronal differentiation (75) in addition to neurite outgrowth (76).

Indole-mediated neurogenic effects correlated with increased VEGF-α. Earlier reports have described a promotion of adult neurogenesis (77, 78), greater hippocampal dendritic arborization (79), and neuroprotective and synaptotrophic effects on lesioned neurons with VEGF-α (80). While it remains unclear which receptors transduce VEGF signals in NPCs and neurons, VEGFR2 (also known as FLK1 or KDR)—which was up-regulated at the gene expression level in indole-treated hippocampi—is critical for VEGF-induced enhancements of hippocampal neuron dendritic arborization (79). While our observations and these additional effects of indole may arise directly through interactions with the AhR in NPCs or NSCs, our data do not exclude effects mediated

![Image](https://doi.org/10.1073/pnas.2021091118)
by AhR-expressing bystander cells, for example, astrocytes (40). Further investigations are needed.

To integrate preexisting circuits and participate in hippocampal functioning, newborn neurons undergo dynamic neurite remodeling resulting in the formation of new synapses (81, 82). Our results demonstrated a profound enhancement of neurite remodeling resulting in the formation of new synapses (81, 82).

Materials and Methods

Animals. All experiments were performed in accordance with institutional guidelines and approved by the Regional Animal Research Ethical Board, Institutional Animal Care and Use Committee, Singapore (protocol nos. AUP-0025 and 2016SHS/1265). To assess the effects of indole on AhR in vivo, adult (10- to 14-wk-old) male mice on a C57BL/6J genetic background were used. GF, SPF, monoassociated adult (10- to 14-wk-old) male mice on a C57BL/6J genetic background were used. C57BL/6J GF mice were colonized with either E. coli全世界和AhR+/- and AhR+/- mice used for experiments, which were maintained under SPF conditions and genotyped from tail samples on weaning at 3 wk old.

Indole administration. Male C57BL/6J mice (8- to 12-wk-old SPF, GF, and MT-E. coli) were randomly assigned regular drinking water or indole-spiked drinking water. Indole (87.6 mg) was dissolved in milliQ water (500 mL, 15 mM) by stirring for 12 h before being filtered twice and diluted in drinking bottles (final concentration of 200 μM). All water was provided ad libitum and changed weekly. No change in water intake or body weight was observed. Mice were euthanized by carbon dioxide inhalation and tissues harvested at 10 or 5 wk time points for Western blot and qRT-PCR and immunohistochemistry analysis, respectively.

Tissue collection. Briefly, following blood collection from the heart and intracardial perfusion with PBS, brains were rapidly removed and cut down the midline. The right side of each brain was fixed in paraformaldehyde (PFA, 4%) for 24 h and cryoprotected in sucrose solution (30%) for 24 h before being frozen in optimal cutting temperature solution (Tissue-Tek). Free-floating cryosections (30 μm) were collected using a freezing microtome (Leica #CM3050S Leica) and stored in cryoprotectant at 4 °C until use. Every 12th section was selected for immunohistostaining. The right half of each brain was micro-dissected in ice-cold PBS and whole hippocampi snap frozen in liquid nitrogen and stored at −80 °C.

Metabolite analysis. Kynurenine and indole-3-acetic acid were quantified using protocols based on previously described methods (88) (SI Appendix, Materials and Methods).

DAB staining, imaging, and newborn neuron quantification. Free-floating brain sections were washed in PBS (0.01 M) and incubated overnight with monoclonal anti-AhR antibody (1:1,000) at room temperature before incubation in biotinylated goat anti-mouse IgG as per the manufacturer’s instructions (1:1,000; Vector Laboratories). Brain sections were mounted on glass slides (Matsunami, MAS-GP, 59901 76 × 26 mm). DCX-positive immunostaining was visualized by the peroxidase method and DAB kit (SK-4100, Vector Laboratories). The quantification of DCX-labeled cells in the DGs was performed as previously described (89) using a Zeiss AxioScan.Z1 slide scanner microscope by a trained researcher blind to the treatment group. Further details are provided in SI Appendix, Materials and Methods.

RNA extraction and quantitative real-time PCR. Total RNA was isolated from whole hippocampi using RNeasy Mini Kit (Qiagen) as per the manufacturer’s instructions. RNA purity was confirmed using a Nanodrop 2000 (Thermo Fisher Scientific) and quality determined by gel electrophoresis and visualized on a Bioanalyzer (Agilent). RNA (500 ng) was reverse transcribed using iScript II Supermix with random Hexamers DNA (50 ng) used for quantitative real-time PCR on a Quantstudio 6 Flex Real-Time PCR system using fast SYBR Green PCR Master Mix (Applied Biosystems) and 0.45 μg oligonucleotide pairs (SI Appendix, Table S1). Samples were run in triplicates, and biological sample sizes are stated in figure legends. Relative gene expressions were normalized to β-actin and computed by the –ΔΔCT method.

Western blotting. Whole hippocampi tissue homogenates (10 μg per lane) were analyzed by Western blot using the following antibodies: anti-PSD-95 (Cell Signaling Technology, #34095), anti-synaptophysin (Cell Signaling Technology, #43295), anti-Apore2 (Cell Signaling Technology, #13144), anti-VGF (Abcam, ab68334), or anti-β-actin (Santa Cruz). Protein band quantification was performed by densitometry analysis against β-actin using ImageJ software (NIH). Further details are provided in SI Appendix, Materials and Methods.

Ex Vivo Studies.

Neurosphere culture. NPCs were isolated from E14.5 mouse forebrains as previously described (90). Briefly, subventricular zones were dissected and the meninges removed before being digested in Accutase cell detachment solution (STEMCELL Technologies). 100 μL, 30 min at 37 °C) followed by mechanical dissociation by trituration with a Pasteur pipette. Single cells were resuspended at 40,000 cells per mL in NPC proliferation medium (EMbyoMax with L-Glutamine, without Heps Dulbecco’s Modified Eagle Medium/F2 (Sigma-Aldrich, Merck)) supplemented with recombinant fibroblast growth factor 20 (20 ng mL−1, Invitrogen), human epidermal growth factor (10 ng mL−1 B-27), and N-2 (1%, 100x) (all growth factors are from Gibco, Thermo Fisher Scientific).
Neurite Tracer functions in ImageJ (Fiji, Image J, NIH). An additional assessment of neurite branching was conducted by manual counting using the ImageJ software. The details of statistical tests used are provided in the figure legends. P values ≤ 0.05 were considered significant.

Data Availability. All study data are included in the article and/or SI Appendix.

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