# JOURNALS IN SCIENCE

## Biological Psychology

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### Blood Pressure Reactivity to Stress Is Better for People Who Recently Had Penile-Vaginal Intercourse Than for People Who Had Other or No Sexual Activity

*Stuart Brody*

Penile-vaginal intercourse (PVI) but not other sexual behavior is associated with better psychological and physiological function. I examined the relationship of sexual behavior patterns to blood pressure and its reactivity to stress (public speaking and verbal arithmetic). For a fortnight, twenty-four women and twenty-two men used daily diaries to record PVI, masturbation, and partnered sexual behavior in the absence of PVI. Persons who reported PVI (but no other sexual activities) had better stress response (less reactivity and/or lower baseline levels) than persons reporting other or no sexual behaviors. Persons who only masturbated or had partnered sex without PVI had 14 mmHg more systolic blood pressure reactivity than those who had PVI but not the other behaviors. Many variables were examined but failed to confound the observed relationships. The magnitude of the sexual behavior effect on blood pressure reactivity is greater than of other factors in the literature. These findings add to the research corpus on the benefits of PVI (differentiated from other sexual activities).

## Cell

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### Identification of Intrinsic Determinants of Midbrain Dopamine Neurons

*Elisabet Andersson et al.*

The prospect of using cell replacement therapies has raised the key issue of whether elucidation of developmental pathways can facilitate the generation of therapeutically important cell types from stem cells. The authors show that the homeodomain proteins Lmx1a and Msx1 function as determinants of midbrain dopamine neurons, cells that degenerate in patients with Parkinson's disease. Lmx1a is sufficient and required to trigger dopamine cell differentiation. An early activity of Lmx1a is to induce the expression of Msx1, which complements Lmx1a by inducing the proneural protein Ngn2 and neuronal differentiation. Importantly, expression of Lmx1a in embryonic stem cells results in a robust generation of dopamine neurons with a "correct" midbrain identity. These data establish that Lmx1a and Msx1 are critical intrinsic dopamine-neuron determinants in vivo and suggest that they may be essential tools in cell replacement strategies in Parkinson's disease.

## Journal of Neuroscience

Volume 26, Number 14
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### Cortical Pain Responses in Human Infants

*Rebeccah Slater et al.*

Despite the recent increase in our understanding of the development of pain processing, it is still not known whether premature infants are capable of processing pain at a cortical level. In this study, changes in cerebral oxygenation over the somatosensory cortex

were measured in response to noxious stimulation, using real-time near-infrared spectroscopy in eighteen infants aged between twenty-five and forty-five weeks postmenstrual age. The noxious stimuli were heel lances performed for routine blood sampling; no blood tests were performed solely for the purpose of the study. Noxious stimulation produced a clear cortical response, measured as an increase in total hemoglobin concentration (HbT) in the contralateral somatosensory cortex, from twenty-five weeks (mean HbT=7.74 micromoles per liter; standard error, 1.10). Cortical responses were significantly greater in awake compared with sleeping infants, with a mean difference of 6.63 micromoles per liter (95% confidence interval [CI] limits: 2.35, 10.91 micromoles per liter; mean age, 35.2 weeks). In awake infants, the response in the contralateral somatosensory cortex increased with age (regression coefficient, 0.698 micromoles per liter per week; 95% CI limits: 0.132, 1.265 micromole per liter per week) and the latency decreased with age (regression coefficient,–0.9861 micromole per liter per week; 95% CI limits: –1.5361, –0.4361 micromole per liter per week; age range, 25–38 weeks). The response was modality specific because no response was detected after non-noxious stimulation of the heel, even when accompanied by reflex withdrawal of the foot. The authors conclude that noxious information is transmitted to the preterm infant cortex from twenty-five weeks, highlighting the potential for both higher-level pain processing and pain-induced plasticity in the human brain from a very early age.

## Nature

Volume 440, Number 7081
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### Expression Profiling in Primates Reveals a Rapid Evolution of Human Transcription Factors

*Yoav Gilad et al.*

Although it has been hypothesized for thirty years that many human adaptations are likely to be due to changes in gene regulation, almost nothing is known about the modes of natural selection acting on regulation in primates. Here the authors identify a set of genes for which expression is evolving under natural selection. They use a new multispecies complementary DNA array to compare steady-state messenger RNA levels in liver tissues within and between humans, chimpanzees, orangutans, and rhesus macaques. Using estimates from a linear mixed model, they identify a set of genes for which expression levels have remained constant across the entire phylogeny (seventy million years), and are therefore likely to be under stabilizing selection. Among the top candidates are five genes with expression levels that have previously been shown to be altered in liver carcinoma. The authors also find a number of genes with similar expression levels among nonhuman primates but significantly elevated or reduced expression in the human lineage, features that point to the action of directional selection. Among the gene set with a human-specific increase in expression, there is an excess of transcription factors; the same is not true for genes with increased expression in chimpanzees.

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### Pluripotency of Spermatogonial Stem Cells from Adult Mouse Testis

*Kaomei Guan et al.*

Embryonic germ cells as well as germline stem cells from neonatal mouse testis are pluripotent and have differentiation potential similar to embryonic stem cells, suggesting that the germline lineage may retain the ability to generate pluripotent cells. However, until now there has been no evidence for the pluripotency and plasticity of adult spermatogonial stem cells (SSCs), which are responsible for maintaining spermatogenesis throughout life in the male. Here the authors show the isolation of SSCs from adult mouse testis using genetic selection, with a success rate of 27 percent. These isolated SSCs respond to culture conditions and acquire embryonic stem cell properties.

The authors name these cells multipotent adult germline stem cells (maGSCs). They are able to spontaneously differentiate into derivatives of the three embryonic germ layers in vitro and generate teratomas in immunodeficient mice. When injected into an early blastocyst, SSCs contribute to the development of various organs and show germline transmission. Thus, the capacity to form multipotent cells persists in adult mouse testis. Establishment of human maGSCs from testicular biopsies may allow individual cell-based therapy without the ethical and immunological problems associated with human embryonic stem cells. Furthermore, these cells may provide new opportunities to study genetic diseases in various cell lineages.

## Nature Biotechnology

Volume 24, Number 4
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### Generation of Cloned Transgenic Pigs Rich in Omega-3 Fatty Acids

*Liangxue Lai et al.*

Meat products are generally low in omega-3 (*n-3*) fatty acids, which are beneficial to human health. The authors describe the generation of cloned pigs that express a humanized *Caenorhabditis elegans* gene, fat-1, encoding an *n-3* fatty acid desaturase. The hfat-1 transgenic pigs produce high levels of *n-3* fatty acids from *n-6* analogs, and their tissues have a significantly reduced ratio of *n-6*/*n-3* fatty acids ($P < 0.001$).

## PNAS: Proceedings of the National Academy of Sciences USA

Volume 102, Number 49
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### Implanted Hair Follicle Stem Cells Form Schwann Cells That Support Repair of Severed Peripheral Nerves

*Yasuyuki Amoh et al.*

The hair follicle bulge area is an abundant, easily accessible source of actively growing, pluripotent adult stem cells. Nestin, a protein marker for neural stem cells, also is expressed in follicle stem cells and their immediate, differentiated progeny. The fluorescent protein GFP, whose expression is driven by the nestin regulatory element in transgenic mice, served to mark the follicle cell fate. The pluripotent nestin-driven GFP stem cells are positive for the stem cell marker CD34 but negative for keratinocyte marker keratin 15, suggesting their relatively undifferentiated state. These cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. In vivo studies show the nestin-driven GFP hair follicle stem cells can differentiate into blood vessels and neural tissue after transplantation to the subcutis of nude mice. Equivalent hair follicle stem cells derived from transgenic mice with beta-actin-driven GFP implanted into the gap region of a severed sciatic nerve greatly enhance the rate of nerve regeneration and the restoration of nerve function. The follicle cells transdifferentiate largely into Schwann cells, which are known to support neuron regrowth. Function of the rejoined sciatic nerve was measured by contraction of the gastrocnemius muscle upon electrical stimulation. After severing the tibial nerve and subsequent transplantation of hair follicle stem cells, walking print length and intermediate toe spread significantly recovered, indicating that the transplanted mice recovered the ability to walk normally. These results suggest that hair follicle stem cells provide an important, accessible, autologous source of adult stem cells for regenerative medicine.

Volume 103, Number 16
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**Neural Mechanisms of Genetic Risk for Impulsivity and Violence in Humans**

*Andreas Meyer-Lindenberg et al.*

Neurobiological factors contributing to violence in humans remain poorly understood. One approach to this question is examining allelic variation in the X-linked monoamine oxidase A (*MAOA*) gene, previously associated with impulsive aggression in animals and humans. The authors have studied the impact of a common functional polymorphism in *MAOA* on brain structure and function assessed with MRI in a large sample of healthy human volunteers. The authors show that the low-expression variant, associated with increased risk of violent behavior, predicted pronounced limbic volume reductions and hyperresponsive amygdala during emotional arousal, with diminished reactivity of regulatory prefrontal regions, compared with the high expression allele. In men, the low expression allele is also associated with changes in orbitofrontal volume, amygdala and hippocampus hyperreactivity during aversive recall, and impaired cingulate activation during cognitive inhibition. Their data identify differences in limbic circuitry for emotion regulation and cognitive control that may be involved in the association of *MAOA* with impulsive aggression, suggest neural systems-level effects of X-inactivation in human brain, and point toward potential targets for a biological approach toward violence.

## Science

Volume 311, Number 5763
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***Cdx2* Gene Expression and Trophectoderm Lineage Specification in Mouse Embryos**

*Kaushik Deb et al.*

Controversy exists as to whether individual blastomeres from two-cell-stage mouse embryos have identical developmental properties and fate. The authors show that the transcription factor Cdx2 is expressed in the nuclei of cells derived from the late-dividing but not the first-dividing blastomere of two-cell embryos and, by lineage tracing and RNA interference knock-down experiments, that this lagging cell is the precursor of trophectoderm. *Cdx2* mRNA is localized toward the vegetal pole of oocytes, reorients after fertilization, and becomes concentrated in the late-dividing, two-cell-stage blastomere. The asymmetrical distribution of *Cdx2* gene products in the oocyte and embryo defines the lineage to trophectoderm.

Volume 311, Number 5768
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**Reversal of Diabetes in Non-obese Diabetic Mice without Spleen-Cell-Derived Beta Cell Regeneration**

*Anita S. Chong et al.*

Autoimmune destruction of beta cells is the predominant cause of type 1 diabetes mellitus in humans and is modeled in nonobese diabetic (NOD) mice. Many therapeutic interventions prevent the development of type 1 diabetes mellitus in NOD mice, but few can induce its reversal once established. Intervention with Freund's complete adjuvant, semi-allogeneic splenocytes, and temporary islet transplantation has been reported to cure NOD mice of established type 1 diabetes mellitus. Using the same approach, the authors report that this treatment cured 32 percent of NOD mice of established diabe-

tes (blood glucose greater than 340 milligrams per deciliter), although beta cells in these mice were not derived from donor splenocytes.

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**Islet Recovery and Reversal of Murine Type 1 Diabetes in the Absence of Any Infused Spleen Cell Contribution**

*Junko Nishio et al.*

A cure for type 1 diabetes will probably require the provision or elicitation of new pancreatic islet beta cells as well as the reestablishment of immunological tolerance. A 2003 study reported achievement of both advances in the NOD [nonobese diabetic] mouse model by coupling injection of Freund's complete adjuvant with infusion of allogeneic spleen cells. It was concluded that the adjuvant eliminated anti-islet autoimmunity and the donor splenocytes differentiated into insulin-producing (presumably beta) cells, culminating in islet regeneration. Here the authors provide data indicating that the recovered islets were all of host origin, reflecting that the diabetic NOD mice actually retain substantial beta cell mass, which can be rejuvenated/regenerated to reverse disease upon adjuvant-dependent dampening of autoimmunity.

Volume 311, Number 5768
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**Immunological Reversal of Auto-immune Diabetes without Hematopoietic Replacement of Beta Cells**

*Anish Suri et al.*

Type 1 diabetes mellitus results from the autoimmune destruction of the beta cells of the pancreatic islets of Langerhans and is recapitulated in the nonobese diabetic strain of mice. In an attempt to rescue islet loss, diabetic mice were made normoglycemic by islet transplantation and immunization with Freund's complete adjuvant along with multiple injections of allogeneic male splenocytes. This treatment allowed for survival of transplanted islets and recovery of endogenous beta cell function in a proportion of mice, but with no evidence for allogeneic splenocyte–derived differentiation of new islet beta cells. Control of the autoimmune disease at a crucial time in diabetogenesis can result in recovery of beta cell function.

## Stem Cells

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**Role of Transcription Factors in Motoneuron Differentiation of Adult Human Olfactory Neuroepithelial-Derived Progenitors**

*Xiaodong Zhang et al.*

Neurosphere-forming cell (NSFC) lines have been established from cultures of human adult olfactory neuroepithelium. Few of these cells ever express mature neuronal or glial markers in minimal essential medium supplemented with 10 percent fetal bovine serum or defined medium. However, these neural progenitors have the potential to differentiate along glial or neuronal lineages. To evaluate the potential of NSFCs to form motoneurons, transcription factors Olig2, Ngn2, and HB9 were introduced into NSFCs to determine if their expression is sufficient for motoneuron specification and differentiation, as has been shown in the early development of the avian and murine central nervous systems in vivo. NSFCs transfected with Olig2, Ngn2, and HB9 alone exhibited no phenotypic lineage restriction. In contrast, simultaneous transfection of Ngn2 and HB9 cDNA increased the expression of Isl1/2, a motoneuron marker, when the cells were maintained in medium supplemented with retinoic acid, forskolin, and sonic hedgehog. Furthermore, a population of Olig2-expressing NSFCs also expressed Ngn2. Cotransfection of NSFCs with Olig2 and HB9, but not Olig2 and Ngn2, increased Isl1/2 expression. Coculture of NSFCs transfected with Ngn2-HB92 or

Olig2 and HB9 with purified chicken skeletal muscle demonstrated frequent contacts that resembled neuromuscular junctions. These studies demonstrate that transcription factors governing the early development of chick and mouse motoneuron formation are able to drive human adult olfactory neuroepithelial progenitors to differentiate into motoneurons in vitro. The authors' long-term goal is to develop cell populations for future studies of the therapeutic utility of these olfactory-derived NSFCs for autologous cell replacement strategies for central nervous system trauma and neurodegenerative diseases.

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**Differentiation of Mouse Embryonic Stem Cells following Rnai-Mediated Silencing of OCT4 and Nanog**

*Shelley R. Hough et al.*

RNAi holds great promise as a tool to study the basic biology of stem cells or to direct differentiation in a specific manner. Barriers to achieving efficient and specific gene silencing in RNAi experiments include limitations in transfection efficiency and in the efficacy and specificity of RNAi silencing effectors. Here the authors combine methods of efficient lipid-mediated delivery with chemically modified RNAi compounds to silence genes related to pluripotency, in order to direct differentiation of mouse embryonic stem cells. Following transfection of embryonic stem cells with OCT4- or Nanog-targeted RNAi compounds, levels of OCT4 or Nanog transcript and protein were reduced accordingly. Reduction in OCT4 expression correlated with induction of trophectoderm genes *Cdx2*, *Hand1*, and *PL-1*, with formation of cells with trophoblast giant cell phenotype after six days. Reduction in Nanog expression correlated with induction of extra-embryonic endoderm genes *GATA4*, *GATA6*, and *laminin B1*, with subsequent generation of groups of cells with parietal endoderm phenotype. The results indicate that transient inhibition of OCT4 or Nanog by RNAi compounds is sufficient to induce differentiation toward extraembryonic lineages, which supports the model that these transcription factors function in a dose-dependent manner to influence cell fate.

## Stem Cells and Development

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**Transplantation of a Novel Cell Line Population of Umbilical Cord Blood Stem Cells Ameliorates Neurological Deficits Associated with Ischemic Brain Injury**

*Jing Xiao et al.*

Umbilical cord blood is a rich source of hematopoetic stem cells (HSCs). The authors have isolated a novel cell line population of stem cells from human umbilical cord blood, which exhibit properties of self-renewal but do not have cell-surface markers that are typically found on HSCs. Analysis of transcripts revealed that these cells express transcription factors Oct-4, Rex-1, and Sox-2, which are typically expressed by stem cells. They refer to these novel cells as nonhematopoietic umbilical cord blood stem cells (nh-UCBSCs). Previous studies have shown that the intravenous infusion of umbilical cord blood cells can ameliorate neurological deficits arising from ischemic brain injury. The identity of the cells that mediate this restorative effect, however, has yet to be determined. The authors postulate that nh-UCBSCs may be a source of the umbilical cord blood cells that can mediate these effects. To test this hypothesis, they intravenously injected one million human nh-UCBSCs into rats forty-eight hours after transient unilateral middle cerebral artery occlusion. Animals in other experimental groups received either saline injections or injections of RN33b neural stem cells. Animals were tested for neurological function before the infusion of nh-UCBSCs and at various time periods afterward using a battery of behavioral tests. In limb placement

tests, animals treated with nh-UCBSCs exhibited mean scores that were significantly better than those of animals treated with RN33b neural stem cells or saline. Similarly, in stepping tests, nh-UCBSC-treated animals again exhibited significantly better performance than the other experimental groups of animals. Analysis of infarct volume revealed that ischemic animals treated with nh-UCBSCs exhibited a 50 percent reduction in lesion volume in comparison to saline-treated controls. Histological analysis of brain tissue further revealed the presence of cells that stained for human nuclei. Some human nuclei-positive cells were also co-labeled for NeuN, indicating that the transplanted cells expressed markers of a neuronal phenotype. Cells expressing the human nuclei marker within the brain, however, were rather scant, suggesting that the restorative effects of nh-UCBSCs may be mediated by mechanisms other than cell replacement. To test this hypothesis, nh-UCBSCs were directly transplanted into the brain parenchyma after ischemic brain injury. Sprouting of nerve fibers from the nondamaged hemisphere into the ischemically damaged side of the brain was assessed by anterograde tracing using biotinylated dextran amine. Animals with nh-UCBSC transplants exhibited significantly greater densities of BDA-positive cells in the damaged side of the brain compared to animals with intraparenchymal saline injections. These results suggest that restorative effects observed with nh-UCBSC treatment following ischemic brain injury may be mediated by trophic actions that result in the reorganization of host nerve fiber connections within the injured brain.