COURSE NOTES: Understanding genetics for improving health outcomes

Course Code: CEUGH

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Course Description

The course is intended to provide the tools that holistic nutritionists can use to deliver personalized healthcare to their clients, catered to the genetic makeup of the client. Genetics plays a very robust role in nutrition, detoxification, weightloss and overall health and wellness. Furthermore, genetics can be used not only to improve the health of the clients but also to develop DNA based nutritional plans that can potentially prevent development of chronic diseases. Course participants will gain knowledge on how genetic information can be used to deliver nutritional plans, weightloss strategies, detoxification plans, hormonal balance plans along with nutritional plans to prevent development of chronic disease such as diabetes and cardiovascular diseases.

IHN has partnered with Anantlife Canada Inc., a leader in clinical grade genetic testing for healthcare providers all over the world, to offer a Certified Genetic Testing Provider Certificate upon successful completion of the course. Successful completion of the course implies that the candidates have received the education and training to not only understand genetic concepts pertaining to diet, nutrition, detoxification, fitness, hormonal health and metabolic disorders but have also been trained on interpretation of the genetic testing reports along with development of a DNA based health plan for better health outcomes.

SESSION 5:

NEUROGENETICS AND UNDERSTANDING / PREDICTING HUMAN BEHAVIOUR – HOW DOES THE GENETICS OF NEUROTRANSMITTER BIOLOGY PLAY A ROLE IN PREDICTING HUMAN BEHAVIOUR? HOW CAN THESE BE APPLIED IN PRACTICE?

Human behavior is controlled by neurotransmitters and genes regulate the generation and breakdown of neurotransmitters along with pathways which are induced when neurotransmitters bind to their receptors. Studies have identified genetic variation in neurotransmitter pathways to regulate all kinds of human behavior including addiction, aggression as well as susceptibility to psychiatric disorders such as depression as well as autism. The readings herein are to provide an extensive understanding of the genetics of neurotransmitter pathways as well as how genetic variation in neurotransmitter pathways regulates human behavior..

FOCUS ON: NEUROTRANSMITTER SYSTEMS

C. Fernando Valenzuela, M.D., Ph.D.; Michael P. Puglia; and Stefano Zucca, M.Sc.

Neurotransmitter systems have been long recognized as important targets of the developmental actions of alcohol (i.e., ethanol). Short- and long-term effects of ethanol on amino acid (e.g., γ-aminobutyric acid and glutamate) and biogenic amine (e.g., serotonin and dopamine) neurotransmitters have been demonstrated in animal models of fetal alcohol spectrum disorders (FASD). Researchers have detected ethanol effects after exposure during developmental periods equivalent to the first, second, and third trimesters of human pregnancy. Results support the recommendation that pregnant women should abstain from drinking—even small quantities—as effects of ethanol on neurotransmitter systems have been detected at low levels of exposure. Recent studies have elucidated new mechanisms and/or consequences of the actions of ethanol on amino acid and biogenic amine neurotransmitter systems. Alterations in these neurotransmitter systems could, in part, be responsible for many of the conditions associated with FASD, including (1) learning, memory, and attention deficits; (2) motor coordination impairments; (3) abnormal responsiveness to stress; and (4) increased susceptibility to neuropsychiatric disorders, such as substance abuse and depression, and also neurological disorders, such as epilepsy and sudden infant death syndrome. However, future research is needed to conclusively establish a causal relationship between these conditions and developmental dysfunctions in neurotransmitter systems. *KEY WORDS: Maternal alcohol exposure; prenatal alcohol exposure; fetal alcohol syndrome disorders; pregnancy; developmental disorders; central nervous system; neurotransmitter systems; amino acids; biogenic amines; animal models*

This article reviews recent research on the short- and long-term effects of developmental ethanol[1] (i.e., alcohol) exposure on brain chemical (i.e., neurotransmitter) systems. The article focuses on studies that were performed with tissue from animal models, including rats, mice, guinea pigs, and primates. It is noteworthy that prenatal development in rats and mice corresponds to the first and second trimesters of human pregnancy, whereas the first week of neonatal life corresponds to the third trimester. In guinea pigs and primates, intrauterine development more closely corresponds to the first, second, and third trimesters of human pregnancy. It also is important to keep in mind that the studies in this research area are quite heterogeneous in several respects, including the timing, duration, and route of ethanol exposure; the levels of ethanol that were achieved in blood; and the techniques used to assess the effects of ethanol exposure. Regarding blood ethanol levels, it should be emphasized that the legal intoxication limit for driving is 0.08 g/dl and that, in some cases, developmental exposures to much higher ethanol levels were required to produce significant effects (see table 1). Ethanol concentrations near 0.4 g/dl are typically lethal in individuals who do not regularly drink significant amounts of ethanol and have not developed tolerance to its depressant effects on brain activity. Therefore, care must be exercised when interpreting the results of studies that have used high concentrations of ethanol. This article first provides background information on neurotransmitter systems and their roles in normal central nervous system development and neurodevelopmental disorders. It then reviews studies on the actions of ethanol on two types of neurotransmitter systems: amino acids and biogenic amines. For the most part, the article reviews research published in the past decade. The reader is referred to more comprehensive review articles for additional information (Berman and Hannigan 2000; Costa et al. 2000; Goodlett et al. 2005; Valenzuela et al. 2008; Weinberg et al. 2008). Figure 1 illustrates some of the mechanisms by which developmental ethanol exposure could impair chemical neurotransmitter systems.

Neurotransmitter Systems and Normal Central Nervous System Development

Efficient communication among large numbers of brain cells (i.e., neurons) is necessary for the normal functioning of the nervous system. A central mechanism of neuronal communication involves the release of neurotransmitters that bind to specialized receptors on the target cell, changing its activity. Although neuropeptides are an important category of neurotransmitters involved in neuronal communication and the developmental actions of ethanol, they are beyond the scope of this article. This article will focus on chemical neurotransmitters, which can be divided into three classes: (1) amino acids (e.g., γ-aminobutyric acid [GABA], glycine, and glutamate); (2) biogenic amines (e.g., serotonin, dopamine, norepinephrine, epinephrine, and histamine); and (3) other (e.g., acetylcholine, adenosine triphosphate, and adenosine). This review focuses on the actions of ethanol on the GABA, glutamate, serotonin, and dopamine neurotransmitter systems in the developing brain. Neurons synthesize these neurotransmitters and package them in vesicles that typically are localized at the ends of projections known as axons. Neuronal activation causes the release of neurotransmitters from the axonal terminals

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[1] The terms ethanol and alcohol are used interchangeably in this article.

Table 1 Examples of Recent Studies on the Effects of Developmental Ethanol Exposure on Neurotransmitter Systems*

Neurotransmitter System	Effect of Developmental Exposure	Exposure Period†	Mode of Ethanol Administration	Blood Ethanol Levels (g/dl)‡	Species	Reference
γ–Aminobutyric acid (GABA)	Premature migration of cortical GABAergic interneurons at E14.5	E0.5–E14.5	Liquid diet	0.025 (maternal)	Mice	(Cuzon et al. 2008)
	Altered plasticity and firing of cerebellar GABAergic neurons (Purkinje) at P15–P20	E0–E21	Drinking water	0.08 (maternal)	Mice	(Servais et al. 2007)
	Decrease in levels of $GABA_A$ receptor $\alpha 5$ subunit at E18 and increased expression in adults	E8	Intraperitoneal injection	Not determined	Mice	(Toso et al. 2006)
	Increase in levels of $GABA_A$ receptor α_1 and $\beta_{2/3}$ subunit in adults	E2–E67	Oral intubation	0.32 (maternal)	Guinea Pigs	(Bailey et al. 2001)
	Decrease in cortical GABAergic (and glutamatergic) neuronal numbers during adolescence	E3–E42/168	Intragastric intubation	0.23 (maternal)	Monkeys	(Miller 2006)
	Impaired brain-derived neurotrophic factor (BDNF)-dependent plasticity of hippocampal GABAergic transmission at P4–P6	P2–P6	Vapor chambers	0.025–0.18 (neonate)	Rats	(Zucca and Valenzuela 2010)
	Delayed GABAergic current maturation in medial septum/diagonal band neurons at P12–P15	P4–P6	Oral intubation	0.28 (neonate)	Rats	(Hsiao et al. 2001)
	Widespread neuronal death at P8 potentially caused by ethanol-induced enhancement of $GABA_A$ receptors (and inhibition of *N*-methyl-D-aspartic acid [NMDA] receptors)	P7	Subcutaneous injection	≥0.2 (neonate)	Rats	(Ikonomidou et al. 2000)
Glutamate	Increase in hippocampal glutamate and NMDA receptor levels at E63	E2–E63	Oral intubation	0.28 (maternal)	Guinea Pigs	(Iqbal et al. 2006)
	Impaired glutamatergic transmission and plasticity in the hippocampus	E2–E67	Oral intubation	0.28 (maternal)	Guinea Pigs	(Richardson et al. 2002)
	Impaired NMDA receptor–dependent activation of extracellular receptor–activated kinase	E0–E21	Voluntary drinking using two bottle choice paradigm	0.08 (maternal)	Mice	(Samudio-Ruiz et al. 2009)
	Decrease in NR2A and NR2B NMDA subunit mRNA in the hippocampus. Increase in NR2A mRNA in the cortex and NR2B in cortex and cerebellum.	E8	Intraperitoneal injection	Not determined	Mice	(Incerti et al. 2010)
	Learning or motor deficits in adult animals that could be prevented by NMDA receptor antagonism during withdrawal	P6 (learning) P1–P8 (motor)	Gastric intubation	0.3–0.4 (learning) 0.22 (motor) (neonate)	Rats	(Lewis et al. 2007; Thomas et al. 2004)
	Impaired hippocampal glutamatergic plasticity at P7–P9	P2–P9	Vapor chambers	0.3–0.4 (neonate)	Rats	(Valenzuela 2010*b*)

Table 1 Examples of Recent Studies on the Effects of Developmental Ethanol Exposure on Neurotransmitter Systems* continued from page 107

Neurotransmitter System	Effect of Developmental Exposure	Exposure Period†	Mode of Ethanol Administration	Blood Ethanol Levels (g/dl)‡	Species	Reference
Glutamate continued	Impaired hippocampal plasticity at P30	P9	Subcutaneous injection	0.2–0.5 (neonate)	Rats	(Izumi et al. 2005)
	Decrease in AMP-activated protein kinase (AMPA) receptor currents in the hippocampus at P18–P27	E3–E20	Intragastric intubation	0.18 (maternal)	Rats	(Wijayawardhane et al. 2007)
	Increase in AMPA receptor function in medial septum/diagonal band neurons at P32–P35	P4–P9	Oral intubation	0.35 (neonate)	Rats	(Hsiao and Frye 2003)
	Decrease in levels and function of mGluR5 in the dentate gyrus of adult animals	E3–E21	Liquid diet	0.07–0.14 (maternal)	Rats	(Galindo et al. 2004)
Serotonin	Decreased serotonin innervation correlated with decreased size in regions targeted by this transmitter at E15–E18	E7–E15/18	Liquid diet	0.07–0.14 (maternal)	Rats	(Zhou et al. 2005)
	Increased incidence in sudden infant death syndrome that correlated with serotonergic abnormalities in the brain stem at 40–90 postconceptional weeks	Unknown	Oral ingestion	Unknown		(Kinney et al. Humans 2003)
	Impaired serotonin-dependent respiratory long-term facilitation of brain stem neurons at P5–P7	E0–E21	Drinking water	0.08 (maternal)	Rats	(Kervern et al. 2009)
	Alterations in serotonergic modulation of hypothalamic–pituitary–adrenal axis	E1–E21	Liquid diet	Not determined	Rats	(Hofmann et al. 2007)
	Presence of a serotonin transporter DNA sequence variation (polymorphism) was associated with increased irritability and stress hormone levels during the neonatal period in animals exposed to ethanol in utero.	E0–E164	Oral ingestion	0.02–0.05 (maternal)	Monkeys	(Kraemer et al. 2008)
Dopamine	Persistent reduction in number of spontaneously active dopaminergic neurons in the ventral tegmental area and substantia nigra of developing and adult offspring	E8–E20	Gastric intubation	0.3 (maternal)	Rats	(Wang et al. 2006)
	Decrease in D1 receptor and dopamine transporter levels	E0–E21 plus lactation	Drinking water and mother's milk	0.08 (maternal)§	Rats	(Barbier et al. 2008)
	Early-gestation ethanol exposure reduced dopaminergic function in adulthood. Middle- to late-gestation exposure heightened dopaminergic function	E0–E50, E50–E135, or E0–E135	Oral ingestion	0.02–0.05 (maternal)	Monkeys	(Schneider et al. 2005)

NOTE:* Only recent studies that used in vivo ethanol exposure paradigms were included. See text for discussion of in vitro studies on the acute effects of ethanol.
†Duration of pregnancy is approximately 21 days in rats and mice, 68 days in guinea pigs, 160–180 days in monkeys, and 280 days in humans. E, embryonic; P, postnatal
‡Legal intoxication limit in the U.S. = 0.08 g/dl. §Levels in nursing pups were not measured but are expected to be significantly lower than maternal levels.

onto branch-like projections in adjacent neurons, which are called dendrites. Dendrites contain small thorn-like protrusions known as dendritic spines. Axonal terminals and dendritic spines meet at specialized points of contact, called synapses, which mediate a significant portion of information exchange between neurons (see figure 1). Neurotransmitter receptors are not only expressed in target cell dendrites but also in axonal terminals, where these receptors regulate neurotransmitter release (see figure 1). Reuptake into the axonal terminal or neighboring support cells (i.e., glial cells) or enzymatic breakdown decreases synaptic levels of the chemical neurotransmitter, terminating its action.

Neurotransmission in the mature central nervous system relies on the proper assembly of synapses during development, a process that requires multiple steps. Development of the nervous system begins with the recruitment of progenitor cells (precursors of neurons and glial cells) into a specialized structure called the neural plate. The neural plate then folds to form the neural tube, which subdivides

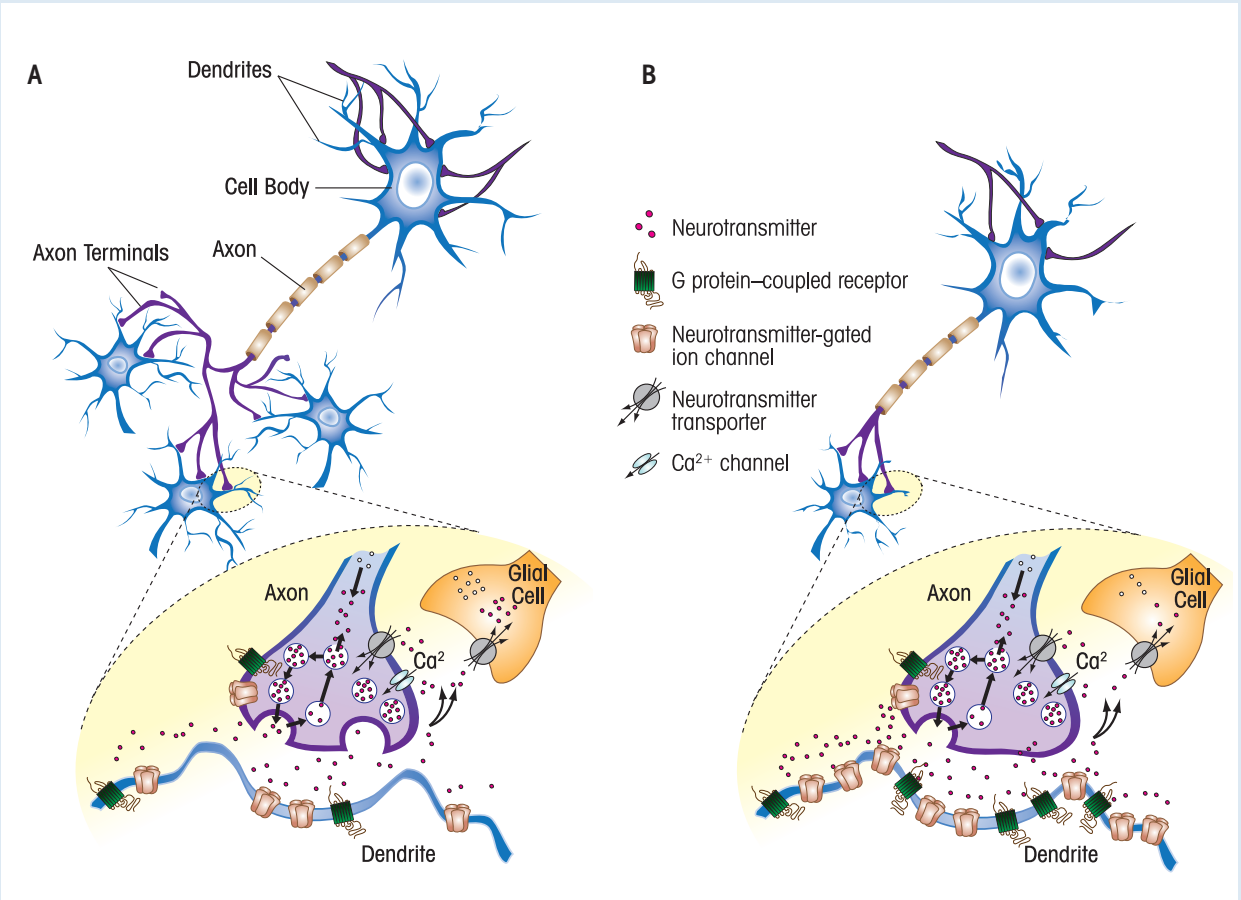

Figure 1 Potential sites of action of developmental ethanol exposure on neurotransmitter systems. **A)** Shown in the upper panel is a schematic representation of the components of a neuron, including the cell body and the ends of projections (axons and axon terminals) that release neurotransmitter onto branch-like projections (dendrites) in adjacent neurons. Note that the axons make synaptic connections with dendrites from three adjacent neurons. Shown in the lower panel is a synapse in more detail. In axonal terminals, neurotransmitters are synthesized and packaged into synaptic vesicles. Release of the neurotransmitter is triggered by influx of Ca^{2+} via voltage-gated Ca^{2+} channels. Neurotransmitter release can be modulated by neurotransmitter-gated ion channels and G protein–coupled receptors expressed in axonal terminals. Neurotransmitters act by activating neurotransmitter-gated ion channels and G protein–coupled receptors expressed in target neurons (either at postsynaptic or extrasynaptic locations). One mechanism by which the action of the neurotransmitter can be terminated is by reuptake into the axonal terminal or neighboring glial cells via neurotransmitter transporters. **B)** Upper panel: developmental ethanol exposure could affect a given neurotransmitter system by decreasing the number of neurons, dendrite and axonal length and/or number, and/or modifying the number and/or efficacy of synapses. Lower panel: developmental ethanol exposure can affect any of the components involved in neurotransmission. In this hypothetical example, ethanol exposure decreased neurotransmitter release, and this led to a compensatory increased in the levels of postsynaptic and extrasynaptic neurotransmitter receptors in the target neuron.

in a complex manner. The anterior and posterior regions of the neural tube ultimately give rise to the brain and spinal cord, respectively. Within the neural tube, progenitor cells are transformed into neuronal and glial cells. Immature neurons migrate to their final locations and gradually acquire axons, dendrites, and synapses. Information exchange between axons and dendrites determines whether synapses are maintained or eliminated, and chemical neurotransmitters play important roles in each of these neuronal developmental steps. Even before synapses are formed, GABA and glutamate regulate progenitor cell proliferation, migration, and differentiation (Manent and Represa 2007). These neurotransmitters could be released from growing axons via reverse action of neurotransmitter transporters that normally pump neurotransmitters into the axonal terminal. In immature neurons, glutamate and GABA contribute to the maturation of dendrites and axons and also are directly involved in the generation and refinement of synaptic contacts (Cline and Haas 2008). Neurotransmitter systems have different properties during development and maturity. For instance, neurons throughout the developing central nervous system generate oscillatory electrical activity that plays a central role in the construction of neuronal networks. These unique properties contribute to the formation and maturation of neuronal circuits and also make immature neuronal networks particularly susceptible to genetic or environmental insults (Ben-Ari 2008).

Role of Alterations in Neurotransmitter Systems in Neurodevelopmental Disorders

Multiple studies have identified neurotransmitter systems as major substrates of neurodevelopmental disorders, including autism, Down syndrome, and fetal alcohol spectrum disorders (FASD). Although these conditions have different causes, they are all characterized by altered neuronal communication that can be explained by underlying deficiencies in synapse development. Ethanol exposure during development has been shown to cause long-lasting defects in both the structure and function of synapses. Several mechanisms could underlie these persistent defects. Death, abnormal migration, or arrested maturation in a population of neurons will deprive their targets from receiving appropriate synaptic inputs, allowing for abnormal synaptic connections to be formed.

Even if synapses are formed properly, ethanol can affect the normal progression of their developmental program, which involves stabilization of functional synapses and pruning of unneeded synapses. Importantly, these synaptic refinement processes are regulated by chemical neurotransmission and require equilibrium (also known as homeostasis) between inhibitory and excitatory influences (Ramocki and Zoghbi 2008). This equilibrium can be altered by genetic defects, such as in the case of Fragile X syndrome, which is characterized by deficits in excitatory synaptic transmission mediated by the amino acid transmitter glutamate; or exposure to toxic agents such as ethanol, which, as discussed below, produces complex effects on the balance between excitatory and inhibitory neurotransmitters. In response to these perturbations, developing neurons attempt to restore equilibrium with compensatory changes that often involve increases or decreases in the function of proteins (e.g., neurotransmitter receptors) involved in chemical neurotransmission. These compensatory changes are not always able to restore equilibrium, and this slows down or accelerates developmental programs, causing abnormal assembly of neuronal circuits and long-lasting alterations in chemical neurotransmission. The precise chain of events leading from developmental insult-induced alterations in neurotransmitter systems to persistent neurochemical alterations during adulthood is currently unknown. In the case of developmental ethanol exposure, this issue is very difficult to study because drinking during pregnancy can occur in many different patterns—for instance, single versus repeated exposure, ingestion of low versus high amounts of ethanol, and exposure during early versus late pregnancy. The studies reviewed below are quite heterogeneous, involving diverse animal models, patterns of developmental ethanol exposure, and study end points. Significant progress has been made in this area of research in recent years, as reviewed below.

GABA

GABA is synthesized from glutamate by the enzyme glutamate decarboxylase and is the main inhibitory transmitter in the mature mammalian brain. Two classes of receptors—the $GABA_A$ and $GABA_B$ receptors—mediate the actions of this neurotransmitter. Most studies related to FASD have focused on $GABA_A$ receptors, which are GABA-activated ion channels that are permeable to chloride ions (Cl^-). When these receptors are activated by GABA in mature neurons, Cl^- flows into the cell making the membrane potential more negative and thereby decreasing excitability of the neuron (see figure 2). However, when these receptors are activated in immature neurons, Cl^- flows out of the cell, making the membrane potential more positive. Therefore, in contrast to its effects on mature neurons, GABA can actually excite immature neurons (see figure 2) (Ben-Ari 2002). These excitatory actions of GABA during development contribute to its involvement in the control of neuronal growth, neuronal migration, and synapse formation/refinement. In rodents, the function of $GABA_A$ receptors switches from excitatory to inhibitory at the end of the period equivalent to the third trimester (i.e., by postnatal days 10 to 12).

Developmental Ethanol Exposure and GABA

Two recent studies highlight the importance of ethanol's actions on the GABA neurotransmitter system during early developmental stages. The first study concerns the effect of ethanol on the generation of new neurons in the

cerebral cortex. Sathyan and colleagues (2007) showed that fetal mouse cerebral cortical progenitor cells exposed to ethanol for 5 days (0.32 g/dl) decreased expression of small noncoding messenger RNA regulatory molecules (microRNAs), and the coordinated effects of ethanol on these microRNAs triggered premature maturation of the progenitor cells. The mechanism of action of ethanol involved, in part, activation of $GABA_A$ receptors in the progenitor cells. The second study addressed the effect of ethanol on neuronal migration, demonstrating that exposing mice to a low concentration of ethanol in utero (see table 1) promoted premature migration of immature GABA interneurons into the cerebral cortex (Cuzon et al. 2008). Studies with cortical slices suggested that ethanol produces premature migration of immature GABAergic interneurons by increasing both ambient GABA levels and $GABA_A$ receptor activation, which could act by stimulating these interneurons (see figure 2). These findings suggest that daily consumption of small amounts of ethanol (such as a glass of wine with meals) during the first and second trimesters of pregnancy could have significant effects on the development of GABAergic neurons in the fetus. Given the prominent role of GABA during development, this could significantly affect the normal development of cortical neuronal circuits. Collectively, these studies emphasize that ethanol can affect the function of the GABA neurotransmitter system even before synapses have been formed and that neurochemical imbalances can have profound consequences on early neuronal development.

During later stages of development, ethanol exposure also affects the maturation of GABAergic transmission. Hsiao and colleagues (2001) showed that administration of ethanol to rat pups via oral intubation during the third trimester–equivalent period (table 1) delays the developmental increase of $GABA_A$ receptor–mediated currents in medial septum/diagonal band neurons, which are involved in modulation of attention, memory, and other cognitive functions. Because these processes are altered in FASD patients, future studies should investigate whether this is a consequence of deficits in the maturation of GABA input to these neurons.

Potent effects of ethanol exposure on GABA transmission during the third trimester–equivalent period also have been documented in the hippocampus—another brain region that is important for learning and memory processes. In a specific population of hippocampal neurons—namely, those located in the CA3 region—a primitive pattern of neuronal network oscillations has been well characterized. These oscillations are driven, in part, by the above-mentioned excitatory actions of $GABA_A$ receptors (see figure 2). Galindo and colleagues (2005) demonstrated that acute ethanol exposure increases GABA release in the CA3 hippocampal region in brain slices from neonatal rats. This effect was produced by ethanol concentrations as low as 0.05 g/dl and ultimately results in an increase

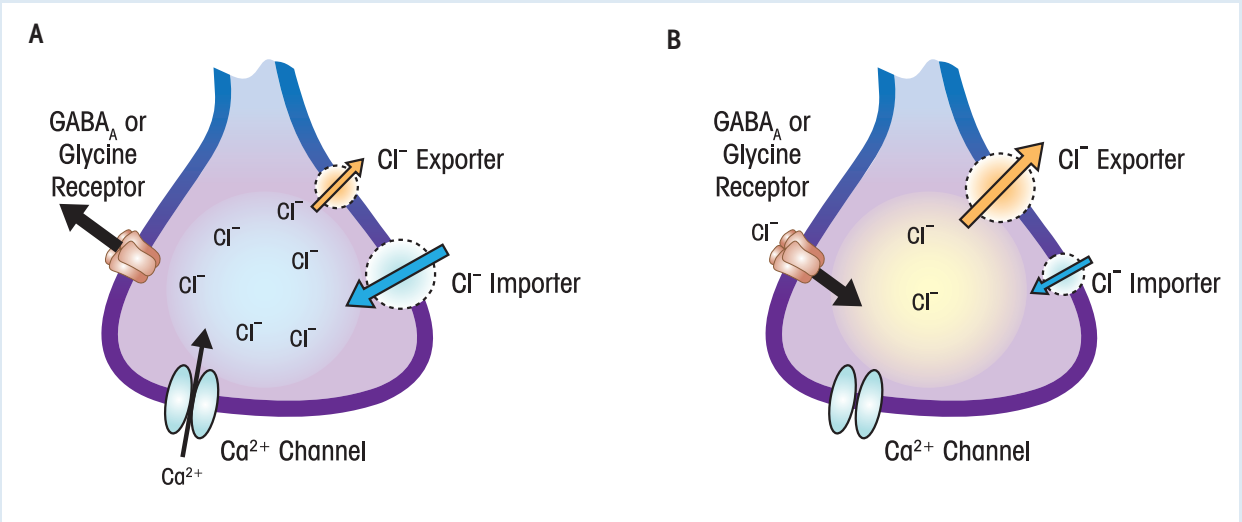

Figure 2 γ-Aminobutyric acid $(GABA)_A$ receptors stimulate immature neurons and inhibit mature neurons. **A)** In immature neurons, intracellular Cl^- concentrations are higher than in mature neurons. This is a consequence of low expression of a Cl^- exporter (potassium/chloride cotransporter type 2; KCC2) and high expression of a Cl^- importer (sodium/potassium/chloride cotransporter type 1; NKCC1). Activation of $GABA_A$ receptors causes Cl^- flux out of the cell, which makes the membrane potential more positive, leading to activation of Ca^{2+} channels. **B)** In mature neurons, intracellular Cl^- concentrations are low. This is a consequence of high expression of a Cl^- exporter and low expression of a Cl^- importer. Activation of $GABA_A$ receptors causes Cl^- flux into the cell, which makes the membrane potential more negative. Ca^{2+} channels are not activated under these conditions. The unique properties of $GABA_A$ receptors during development make them especially vulnerable to ethanol (see text).

in neuronal oscillations. Whether ethanol also affects the switch in the actions of the $GABA_A$ receptors from excitatory to inhibitory currently is under investigation.

Given that oscillatory network activity is thought to be important for the maturation of neuronal circuits in the hippocampus and other brain regions of several animal species including humans (Moody and Bosma 2005), it is possible that this effect of ethanol impairs the formation and/or refinement of synapses even when ethanol is consumed at low levels during late pregnancy. The oscillations control neuronal development by triggering changes in the activity of genes and/or inducing the release of trophic factors (from the Greek *trophe*, to nourish) that stabilize neuronal connections (Mohajerani and Cherubini 2006).

A recent study by Zucca and Valenzuela (2010) characterized the effect of ethanol on the release of trophic factors in the CA3 hippocampal region. Stimulation of pyramidal-shaped neurons in this region causes the dendritic release of a protein known as brain-derived neurotrophic factor (BDNF), which induces a long-lasting enhancement (i.e., potentiation) of GABA transmission that is thought to be essential for the maturation of GABA inputs to these neurons (Gaiarsa 2004). The researchers found that acute exposure to ethanol inhibits this BDNF-mediated potentiation of GABA transmission in hippocampal slices. This effect was very potent, reaching significance at concentrations as low as 0.025 g/dl, and was mediated by inhibition of the L-type category of voltage-gated calcium ion (Ca^{2+}) channels that trigger the dendritic release of BDNF. This effect also was observed after repeated in vivo exposures to low doses of ethanol. Inhibition of this BDNF-dependent form of synaptic potentiation is likely to have a deleterious effect on the maturation of inhibitory circuits in the CA3 hippocampal region, causing an imbalance between excitatory and inhibitory synaptic transmission, and ultimately resulting in alterations in learning, memory, and other cognitive processes.

Developmental ethanol-induced alterations of GABA functioning also can produce long-lasting changes in neuronal circuits by inducing neuronal death. Exposure to high levels of ethanol (≥0.2 g/dl) during the third trimester–equivalent period was shown to cause widespread neuronal death in rats (Ikonomidou et al. 2000). This effect partially was mimicked by the administration of drugs such as barbiturates that enhance $GABA_A$ receptor function and the researchers hypothesized that ethanol triggered cell death

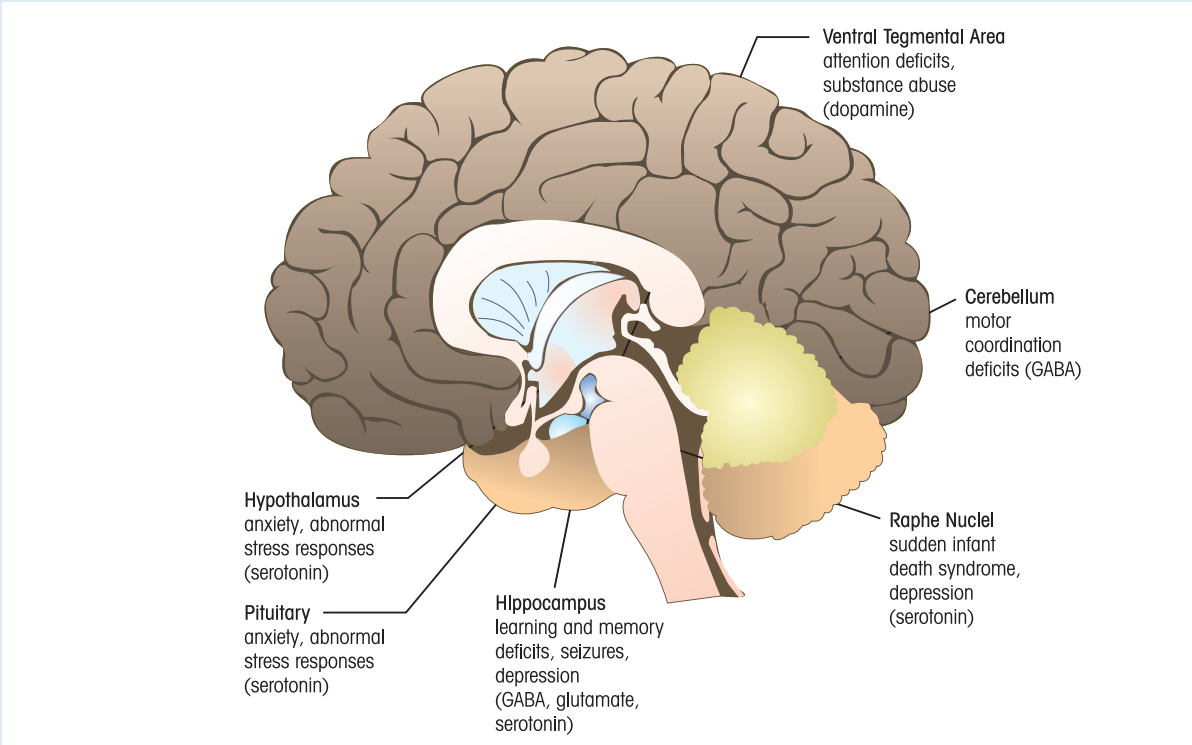

Figure 3 Examples of brain regions where chemical neurotransmitter system alterations have been demonstrated in models of fetal alcohol spectrum disorders (FASD). Shown is a schematic representation of the mature human brain. The potential FASD-linked conditions that could be explained by neurotransmitter system alterations are given under the label for each region. Anxiety and abnormal stress responses (serotonin) apply both to hypothalamus and pituitary. Examples of neurotransmitters that could potentially be involved in these deficits are given in parenthesis.

by inducing excessive inhibition of neuronal activity via $GABA_A$ receptor potentiation. However, Sanderson and colleagues (2009) failed to demonstrate that ethanol directly enhances $GABA_A$ receptor–mediated inhibition of cortical neurons in slices from neonatal rats, which is surprising given that these neurons were shown to be particularly sensitive to ethanol-induced cell death (Ikonomidou et al. 2000).

Persistent alterations in the GABA system could be responsible for behavioral abnormalities observed in the adult offspring from animals exposed to single or multiple doses of ethanol during development. However, the mechanisms responsible for these effects are presently unknown, including the possible connection between these persistent changes and any of the above-described effects of ethanol on the developing GABA neurotransmitter system. In one study of guinea pigs repeatedly exposed to high ethanol concentrations throughout pregnancy, researchers found persistent alterations in GABA transmission (see table 1) and increased levels of certain $GABA_A$ receptor subunits (α_1 and/or $\beta_{2/3}$) in the cerebral cortex and hippocampus of adult offspring (Bailey et al. 2001). This effect could have occurred in response to reduced numbers of GABA neurons or a decrease in the enzymes essential to GABA synthesis (Bailey et al. 2001, 2004). Similarly, elevated levels of the α_5 $GABA_A$ receptor subunit were found in the brains of adult mice exposed to a single ethanol dose during gestational day 8 (neonatal ethanol levels expected to be near 0.5 g/dl) (Toso et al. 2006; Webster et al. 1983). $GABA_A$ receptors containing the α_5 subunit are expressed outside the synaptic area (see figure 1) and exert a persistent inhibitory control on neurons in the hippocampus and other brain regions, including the cerebral cortex (Pirker et al. 2000).

A study of adolescent monkeys exposed to ethanol in utero found reduced numbers of GABA neurons in the cerebral cortex (see table 1) (Miller 2006). Persistent dysfunction and/or loss of GABA neurons could, in part, be responsible for the increased susceptibility to epilepsy that has been linked to FASD (Bell et al. 2010; Bonthius et al. 2001). In addition, alterations in the function of a subtype of GABA neuron (i.e., the Purkinje neuron) in the cerebellum, a brain region important for motor coordination (see figure 3), could contribute to motor deficits observed in FASD patients (Green 2004; Hsiao et al. 1999; Servais et al. 2007). Collectively, these studies indicate that the GABA neurotransmitter system is an important target of developmental ethanol exposure and that further investigation of the mechanisms of action of ethanol on this system is warranted.

Glutamate

Glutamate is the main excitatory neurotransmitter in the mammalian brain and is locally synthesized from glucose. It binds to two classes of receptors: glutamate-gated ion channels and G protein–coupled receptors, which regulate a variety of intracellular signaling pathways via activation of G proteins (i.e., guanine nucleotide–binding proteins). There are three families of glutamate-gated ion channels, named for the compounds that were used to initially identify these channels: (1) the *N*-methyl-D-aspartate (NMDA) receptor, (2) the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor, and (3) the kainate receptor (AMPA and kainate receptors are collectively known as non-NMDA receptors). There also are three families of G protein–coupled glutamate receptors, and these are denoted as group I, II, and III metabotropic glutamate receptors (mGluR I–III), based on their functional properties and effects on neuronal function.

Developmental Ethanol Exposure and the Glutamate Receptors

Short-Term Effects. A significant amount of research in recent years has focused on whether NMDA receptors in developing and mature neurons have the same sensitivity to ethanol. Interest in this issue was kindled, in part, by controversial findings that administration of NMDA receptor blockers during the third trimester–equivalent period triggers neuronal degeneration in many areas of the rat brain. As discussed above, drugs that potentiate $GABA_A$ receptors and ethanol produced a similar effect (Ikonomidou et al. 2000. For a detailed discussion of these findings, see Sanderson et al. 2009.) Given that numerous studies have shown that ethanol inhibits NMDA receptor function in mature neurons, it was hypothesized that ethanol acted, in part, by also inhibiting these receptors in immature neurons throughout the brain. Evidence supporting this hypothesis was found in a study with CA1 hippocampal neurons in slices from neonatal rats, where acute exposure to ethanol concentrations (0.18 to 0.36 g/dl) that are near those required to trigger neuronal degeneration (≥0.2 g/dl) inhibited NMDA receptor–mediated synaptic responses (Puglia and Valenzuela 2010*a*). However, acute exposure to ethanol (0.32 g/dl) did not affect NMDA receptor–mediated currents in another neuronal population that was shown to be particularly sensitive to ethanol-induced cell death: neocortical layer II/III neurons from neonatal rats (Sanderson et al. 2009).

Mameli and colleagues (2005) also have reported finding no direct, acute inhibitory action of ethanol on NMDA receptors in another population of developing rat hippocampal neurons (CA3 pyramidal neurons). The study found that ethanol sensitivity in these receptors is gradually acquired after the third trimester–equivalent period has been completed. The researchers did report that ethanol directly inhibits AMPA receptors in these neurons during the third trimester–equivalent period and that this sensitivity gradually disappears as development progresses. NMDA and AMPA receptors have distinct

subunit compositions during development and maturity, and this could explain the differences in ethanol sensitivity that were observed. In addition, this study found that ethanol can indirectly affect the function of both AMPA and NMDA receptors in developing neurons by decreasing glutamate release; this effect was detected during the third trimester–equivalent period and was mediated by inhibition of the N-type of Ca^{2+} channels that mediates glutamate release onto immature but not mature CA3 pyramidal neurons. Taken together, these findings suggest that if direct inhibition of NMDA receptors by ethanol damages developing neurons, this mechanism cannot be generalized to all brain regions.

An alternative possibility is that inhibition of NMDA receptors during ethanol exposure does not injure developing neurons but that damage actually occurs during ethanol withdrawal and that compensatory increases in NMDA receptor levels and/or function result in excessive ion flux through this channel, which is known to trigger neuronal toxicity. This model is supported by independent studies in which ethanol-induced long-lasting learning deficits were shown to be prevented by administration of NMDA receptor antagonists during withdrawal from exposure to high ethanol levels (see table 1) in models of third trimester–equivalent, binge-like ethanol consumption (Lewis et al. 2007; Thomas et al. 2004).

As mentioned above with respect to GABA, synaptic potentiation mechanisms play a central role in synapse stabilization during the third trimester–equivalent period, and this also applies to glutamatergic synapses (Hanse et al. 2009). Researchers have hypothesized that ethanol could alter the maturation of glutamatergic circuits by interfering with synaptic potentiation mechanisms (Medina and Krahe 2008). Initial studies (Mameli and Valenzuela 2006; Valenzuela et al. 2008) focused on the early portion of the third trimester–equivalent period found that acute exposure to ethanol caused long-lasting potentiation of AMPA receptor-mediated transmission in the CA1 hippocampal region of neonatal rats via the local production and/or release of a steroid-like molecule. However, this potentiating effect of ethanol was not observed under all conditions; acute ethanol exposure was subsequently found to inhibit CA1 AMPA receptor–mediated responses using a different experimental approach (Puglia and Valenzuela 2009). Moreover, this study also found that repeated in vivo ethanol exposure did not affect glutamatergic transmission in the CA1 region. Further research examined this issue during the late portion of the third trimester–equivalent period; acute exposure to high concentrations of ethanol (0.36 g/dl) impaired synaptic potentiation in the CA1 region, indicating that the mechanism of action of acute ethanol exposure involves, at least in part, inhibition of the function of both NMDA receptor– and AMPA receptor–mediated synaptic responses (Puglia and Valenzuela 2010*a*). Importantly, glutamatergic synaptic potentiation also was impaired in neonatal rats repeatedly exposed to high ethanol levels during the third trimester–equivalent period (see table 1) (Puglia and Valenzuela, 2010*b*). Interestingly, in this in vivo study, neither AMPA nor NMDA receptor function was affected by repeated ethanol exposure, suggesting that acute and chronic ethanol exposure impair synaptic potentiation via different mechanisms. Collectively, these studies indicate that synaptic potentiation mechanisms in glutamatergic neuronal circuits are important targets of the short- and long-term actions of developmental ethanol exposure. Future studies should examine whether ethanol-induced impairments of these mechanisms alter maturation of hippocampal circuits and contribute to the long-lasting learning and memory deficits associated with FASD.

Long-Term Effects. In comparison to the action of ethanol on glutamatergic receptor function during development, more studies have examined the long-lasting effects of ethanol on NMDA receptors because they are involved in cellular mechanisms that are thought to be important for learning and memory. NMDA receptor–dependent long-term potentiation of AMPA receptor–mediated synaptic responses may underlie these processes and has been shown to be impaired in FASD (reviewed in Berman and Hannigan 2000). The NMDA receptor typically is formed by two NR1 subunits and two NR2 subunits. A number of studies carried out between 1988 and 1999 (reviewed in Costa et al. 2000) demonstrated that developmental ethanol exposure produces long-term changes in NMDA receptor levels in several brain regions. In the past decade, antibodies that selectively recognize specific NMDA receptor subunits have become widely available, and these have been used in several laboratories to further investigate the developmental effects of ethanol on NMDA receptor expression. These studies have yielded complex results that depend on the method of ethanol administration, dose of ethanol, brain region examined, experimental technique used to measure subunit levels, animal species, and animal age. Researchers have found increases, decreases, and no change in NMDA and/or AMPA receptor subunit levels (for examples see Dettmer et al. 2003; Honse et al. 2003; Naassila and Daoust 2002; Samudio-Ruiz et al. 2010). Long-lasting increases in NMDA receptor levels, coupled with elevated glucocorticoid and glutamate levels, could cause hippocampal damage, as suggested by a study with near-term fetal guinea pigs that were exposed throughout pregnancy to a high concentration of ethanol (see table 1) (Iqbal et al. 2006).

Long-lasting potentiation and other forms of synaptic plasticity (including long-term depression, the counterpart of long-lasting potentiation) can be impaired by certain patterns of developmental ethanol exposure, and this also could be a consequence of persistent alterations in NMDA receptor levels and/or function, including deficits in activation of NMDA receptor–dependent intracellular signaling pathways (Izumi et al. 2005; Margret et al. 2006; Medina and Krahe 2008; Richardson et al. 2002). For instance, NMDA receptor–dependent activation of extracellular receptor–activated kinase, a key enzyme that relays signals

from the cell membrane to the nucleus during long-term synaptic potentiation, recently was shown to be impaired in adult mice exposed throughout pregnancy to relatively low levels of ethanol (see table 1) (Samudio-Ruiz et al. 2009). The mechanisms responsible for these long-lasting effects of developmental ethanol exposure presently are unknown—including whether there is a connection between these and the developmental actions of ethanol on glutamatergic transmission—and none of these studies has conclusively linked these NMDA receptor alterations with behavioral deficits in animal models of FASD.

Long-lasting changes in AMPA receptor function also have been detected in a few recent studies. Increases in AMPA receptor function were detected in medial septum/diagonal band neurons from juvenile rats exposed to ethanol in a binge-like fashion during the third trimester–equivalent period (see table 1) (Hsiao and Frye 2003). Decreases in frequency and amplitude of spontaneous AMPA receptor–mediated currents were found in CA1 hippocampal pyramidal neurons from 18- to 27-day-old rats exposed to ethanol in utero, and this effect was ameliorated by postnatal administration of an agent that stimulates AMPA receptor function (i.e., aniracetam) (see table 1) (Wijayawardhane et al. 2007, 2008). Importantly, this aniracetam treatment regimen reversed learning and memory deficits that were present in untreated rats when they reached 40 days of age (Vaglenova et al. 2008). Clearly, further research efforts should focus on the role of AMPA receptors in the learning disabilities associated with FASD.

Metabotropic GluRs are powerful modulators of synaptic transmission in many brain regions, including the regulation of synaptic potentiation. Metabotropic GluRs have been implicated in learning and memory processes, as well as a human intellectual disabilities (Fragile X syndrome), where the absence of a protein encoded by the Fragile X mental retardation 1 (FMR1) gene causes dysregulation of mGluR-dependent signalling (Bassell and Warren 2008). Metabotropic GluRs also are known to regulate proliferation, differentiation, and survival of neuronal progenitor cells (Catania et al. 2007). Despite the importance of mGluRs for neuronal development, only a handful of studies have examined their role in FASD, and these have only focused on the long-term effects of ethanol. Queen and colleagues (1993) found a reduction in mGluR function in the hippocampus of adult offspring from rats exposed to ethanol during pregnancy (at a blood ethanol level of 0.08 g/dl). Similar findings were reported in another study with rats exposed to ethanol during gestational days 12 to 20 (blood ethanol levels not reported) (Noble and Ritchie 1989). In contrast, Valles and colleagues (1995) found that when rat dams were exposed to ethanol throughout gestation (maternal blood ethanol levels ~0.1 g/dl) as well as during the lactation period (neonatal blood ethanol levels reported as very low), there was an increase in mGluR function in the hippocampus of juvenile offspring. More recently, Galindo and colleagues (2004) showed that levels and function of a specific mGluR subunit (mGluR5) were decreased in part of the hippocampus (i.e., dentate gyrus) of adult offspring of rats gestationally exposed to ethanol (see table 1). In another study, the long-term effects of ethanol were assessed in cultured cerebellar neurons obtained from embryonic day 20 rat fetuses. Exposure of these neurons to 0.15 g/dl of ethanol for 9 to 11 days decreased mGluR function; however, upon 1 day of withdrawal, these responses were enhanced (Netzeband et al. 2002). These studies indicate that mGluRs are important targets of ethanol exposure during development and future research should investigate the role of these receptors in the behavioral abnormalities associated with FASD.

Serotonin

This neurotransmitter, also known as 5-hydroxytryptamine (5-HT), is synthesized from tryptophan by the enzymes tryptophan hydroxylase and aromatic amino acid decarboxylase. It binds to two types of receptors: serotonin-gated ion channels (5-HT_3 receptors) or G protein–coupled receptors (5-HT_1, 5-HT_2, and $5\text{-HT}_{4–7}$ receptors). The body of most serotonin neurons is located in a series of nuclei—known as the raphe nuclei—that are shaped like a seam and are located in the brain stem (see figure 3). Some serotonergic projections from these nuclei descend to the spinal cord where they modulate pain transmission. Other projections ascend to brain regions such as the cortex, hippocampus, and hypothalamus (see figure 3). In the mature brain, the serotonin neurotransmitter system is involved in the regulation of mood, attention, appetite, sleep, and other functions. Alterations in this neurotransmitter system have been linked to neuropsychiatric conditions, including depression. Serotonin is removed from synapses via reuptake mediated by transporters in the axon terminals, which are inhibited by antidepressant medications such as fluoxetine (Prozac®). Serotonin neurons are expressed early in development (embryonic day 12 in rodents), and serotonin released from these neurons has been shown to control progenitor cell proliferation, differentiation, migration, and synapse formation (Frederick and Stanwood 2009). Therefore, the role of alterations in this transmitter system in FASD has been investigated in several laboratories, as these alterations could have a wide impact on neuronal circuit development across different brain regions.

Developmental Ethanol Exposure and Serotonin

Goodlett and colleagues (2005) recently reviewed the effects of developmental ethanol exposure on the serotonergic neurotransmitter system, and the reader is referred to this article for more details. Briefly, studies have shown that developmental ethanol exposure, particularly when it involves exposure during the first and/or second trimester

equivalents, decreases differentiation, migration, and axonal outgrowth of serotonin neurons. The mechanism underlying these effects may involve alterations in cross-talk between glial and neuronal cells. Serotonin neurons release serotonin onto glial cells, activating 5-HT_{1A} receptors expressed in these cells and inducing them to release neurotrophic factors (for instance, a protein known as S100β) that feed-back onto serotonin neurons to nurture them. Activators of 5-HT_{1A} receptors and some neuroprotective peptides have been shown to ameliorate the effects of ethanol on serotonin neuron development (Druse et al. 2005; Zhou et al. 2008).

Several recent studies have investigated the impact of serotonin deficits induced by developmental ethanol exposure. Zhou and colleagues (2005) found that decreased serotonin innervation correlated with reduced numbers of thalamocortical fibers, as well as decreased size of regions targeted by serotonin axons, including the hypothalamus, cerebral cortex, and hippocampus (see figure 3). Kinney and colleagues (2003) discovered a link among deficits in the brain stem serotonin system, prenatal ethanol exposure, prenatal nicotine exposure, and sudden infant death syndrome in autopsy cases from Native American Indians from the Northern Plains. Prenatal ethanol exposure is a major risk factor for sudden infant death syndrome, and this may be a consequence of ethanol–induced abnormalities in the maturation of serotonin neurons in the brain stem, where these neurons play an important role in the control of respiration, heart function, and blood pressure. In agreement with these human studies, developmental ethanol exposure in rats was shown to alter respiratory long-term facilitation, a serotonin-dependent protective mechanism that takes place in brain stem neurons in response to repeated events of low oxygenation (Kervern et al. 2009). In animals that are chronically exposed to ethanol, low oxygenation paradoxically induced long-term depression in these neurons in response to low oxygenation. Application of a 1-μM concentration of the serotonin analog, α-methyl-serotonin, induced respiratory long-term facilitation and depression in control and ethanol groups, respectively. These data are consistent with the model that prenatal ethanol exposure–induced alterations on serotonin modulation of brain stem neurons that control respiration could explain the high incidence of sudden infant death syndrome in FASD. Hofmann and colleagues (2007) observed complex gender-dependent effects on serotonergic modulation of the hypothalamic–pituitary–adrenal axis in adult rat offspring repeatedly exposed to ethanol during prenatal development. This axis is part of the neuroendocrine system and controls stress responses among other important physiological processes (see figure 3). Moreover, Kraemer and colleagues (2008) reported evidence suggesting an interesting interaction between repeated prenatal ethanol exposures and a variation (DNA polymorphism) in the serotonin transporter gene. They found that prenatal ethanol exposed monkeys carrying this serotonin gene variation were more irritable as neonates and exhibited increased stress hormone levels. For more details on the role of serotonin and other neurotransmitters in alterations of stress responses by developmental ethanol exposure, which can be responsible for many of the alterations associated with FASD, including depression, anxiety, learning and memory deficits, and increased susceptibility to infections, see Lee and colleagues (2008) and Weinberg and colleagues (2008).

As mentioned above, alterations in the serotonin neurotransmitter system play a role in depression, and this mood disorder often is present in FASD patients (O'Connor and Paley 2006). Depressive-like behavior also has been detected in rodent models of FASD, and this could be a consequence of reductions in levels of BDNF (see GABA section above) (Caldwell et al. 2008; Castrén and Rantamäki 2008). Antidepressant medications that act by inhibiting serotonin uptake have been shown to restore BDNF levels, suggesting that serotonin controls BDNF production by neurons (Martinowich and Lu 2008). It is therefore possible that deficits in serotonin transmission induced by developmental ethanol exposure result in long-lasting changes in BDNF levels and that this is, in part, responsible for the increased incidence of depression in FASD patients. Future studies should test this possibility.

Dopamine

This biogenic amine transmitter is synthesized from tyrosine by the enzymes tyrosine hydroxylase and L-aromatic amino acid decarboxylase. Dopamine binds to five types of G protein–coupled receptors that are grouped in two families: D_1-like receptors (D_1 and D_5 receptors) and D_2-like receptors (D_2, D_3, and D_4 receptors). The action of dopamine is terminated by reuptake into axonal terminals via dopamine transporters. The cell bodies of dopaminergic neurons are located in the hypothalamus and in brain stem regions known as the substantia nigra pars compacta and the ventral tegmental area (see figure 3). Dopaminergic fibers project extensively throughout many brain regions, including the cortex, hippocampus, and striatum. In the mature brain, dopamine is involved in the regulation of movement, attention, motivation, and reward. Alterations in this neurotransmitter system have been linked to neurological disorders, such as Parkinson's disease (caused by degeneration of substantia nigra pars compacta dopaminergic neurons), as well as neuropsychiatric conditions such as schizophrenia, attention deficit disorder, and substance abuse. In the developing brain, dopamine regulates neuronal differentiation, migration (including that of GABAergic neurons), and axonal and/or dendritic growth (Frederick and Stanwood 2009).

Developmental Ethanol Exposure and Dopamine

Although it is well established that the dopaminergic system is an important target of the developmental actions of

substances of abuse and environmental toxins (Frederick and Stanwood 2009; Thompson et al. 2009), comparatively little is known about the actions of ethanol on this neurotransmitter system.

FASD is associated with attention deficits and increased susceptibility to substance abuse, and this could be the result of alterations in the dopaminergic neurotransmitter system. Studies carried out in the 1980s and 1990s demonstrated that repeated prenatal ethanol exposure decreases dopamine content and turnover, reduces D1 receptor levels, distorts the shape of dopaminergic neurons, and affects sensitivity of dopamine receptors to pharmacological agents (see Wang et al. 2006 and references therein). More recently, a positron emission tomography study detected complex alterations in the dopaminergic neurotransmitter system in young adult Rhesus monkeys that were exposed to low levels of ethanol during different phases of pregnancy (see table 1) (Schneider et al. 2005). Other studies (see Wang et al. 2006 and references therein) have demonstrated that prenatal ethanol exposure persistently reduces the number of spontaneously active dopamine neurons in the ventral tegmental area and substantia nigra of developing and adult offspring (see table 1). The mechanism by which ethanol produces this effect is unknown, but it appears to involve inactivation of a population of dopaminergic neurons in these regions. Importantly, methyphenidate (Ritalin®), a drug used to treat attention deficit disorders, restores normal dopaminergic neuronal activity for a prolonged period of time in rats exposed to ethanol prenatally (Shen and Choong 2006). The precise mechanism by which methylphenidate produces this effect is presently unknown.

In addition to mediating FASD-related attention deficits, alterations in the dopaminergic neurotransmitter system also could underlie the increased incidence of ethanol abuse and other substance abuse disorders that has been observed in individuals exposed to ethanol in utero as well as in animal models of FASD (Alati et al. 2006; Baer et al. 2003; Matta and Elberger 2007; Yates et al. 1998). Barbier and colleagues (2008, 2009) found that exposure of rats to ethanol during pregnancy and lactation increased ethanol consumption and sensitivity to its anxiety-decreasing effects in adult offspring (see table 1). These animals also exhibited increased sensitivity to the rewarding effects of cocaine and increased sensitization to cocaine and amphetamine (these drugs act in part by increasing levels of dopamine in the synapses). Decreases in D_1 and/or dopamine transporter levels were detected in the striatum of these rats. Youngentob and Glendinning (2009) recently reported that ethanol tasted and smelled better to the adolescent offspring of rats exposed to ethanol during pregnancy, resulting in increased ethanol intake. Although the mechanism of this effect is not fully understood, it may involve alterations in the levels of neurotransmitter genes in the olfactory bulb, including levels of the D_2 receptor gene (Middleton et al. 2009). Future studies will be needed

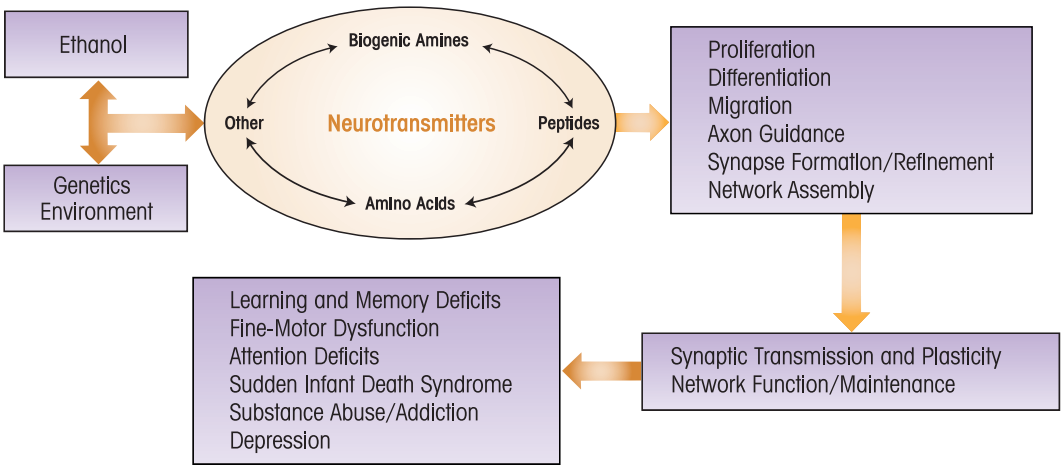

Figure 4 Diagram illustrating the potential role of neurotransmitter system alterations in fetal alcohol spectrum disorders (FASD). Ethanol exposure during development, acting in conjunction with genetic susceptibility factors (for instance, variations in the serotonin transporter gene) and environmental factors (for example, coexposure to nicotine), disrupts the actions of neurotransmitter systems (i.e., biogenic amines, etc) that normally interact in a complex manner to regulate the key processes involved in brain development (i.e. proliferation, etc). Disruption of these processes results in persistent alterations in synaptic transmission/plasticity and neuronal network function. These alterations likely underlie the deficits associated with FASD (i.e., learning and memory deficits). The precise chain of events leading from developmental ethanol exposure to these deficits remains to be determined.

to determine if there is a link between these alterations in D_2 receptor expression and changes in olfactory ethanol sensitivity.

Conclusion

Research on the effects of developmental ethanol exposure on chemical neurotransmitter systems has significantly increased over the past decade. Studies have convincingly demonstrated that neurotransmission in the developing brain does not always respond to ethanol as in the adult brain and that components of developing neurotransmitter systems have unique properties that make them particularly sensitive to the adverse actions of ethanol, even at low levels of exposure. Ethanol-induced abnormalities in the formation and refinement of developing neuronal circuits are likely to be, in part, responsible for the persistent structural and functional brain deficits that characterize FASD. These deficits are ultimately responsible for the behavioral and cognitive alterations present in patients with this disorder and for their increased propensity to have comorbid neuropsychiatric diseases and some neurological disorders (see figures 3 and 4). Future studies should continue to investigate the mechanisms by which ethanol affects amino acid and biogenic amine neurotransmitter systems, and extend this work to other neurotransmitter systems, including peptide neurotransmitters. It is important to also continue to investigate the effects of developmental ethanol exposure on modulators of neurotransmission, as these may be key targets for the development of effective therapeutic interventions against FASD. ■

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REVIEW ARTICLE

Genetics of human aggressive behaviour

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Abstract A consideration of the evolutionary, physiological and anthropological aspects of aggression suggests that individual differences in such behaviour will have important genetic as well as environmental underpinning. Surveys of the likely pathways controlling the physiological and neuronal processes involved highlight, as obvious targets to investigate, genes implicated in sexual differentiation, anxiety, stress response and the serotonin neurotransmitter pathway. To date, however, association studies on single candidates have provided little evidence for any such loci with a major effect size. This may be because genes do not operate independently, but function against a background in which other genetic and environmental factors are crucial. Indeed, a series of recent studies, particularly concentrating on the serotonin and norepinephrine metabolising enzyme, monoamine oxidase A, has emphasised the necessity of examining gene by environmental interactions if the contributions of individual loci are to be understood. These findings will have major significance for the interpretation and analysis of data from detailed whole genome association studies. Functional imaging studies of genetic variants affecting serotonin pathways have also provided valuable insights into potential links between genes, brain and aggressive behaviour.

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Evolutionary origins of aggression

The various facets of human aggression can be considered as evolutionary advantageous traits and as important predictors of success in a competitive modern world; they may also provide an impediment to social cohesion. It is not difficult to imagine how aggression, particularly for males in animal communities, provides a competitive edge in securing resources and in intra-sexual competition via combat. High aggression may even compensate for lack of physical prowess in establishing hierarchy and dominance with respect to reproductive success. Aggression in females can provide protection of their offspring against a range of threats. Such behaviour also has negative attributes and in certain circumstances represents a high-risk strategy with associated possibilities of injury or death. Given such a high likelihood of both potentially positive and negative selective discrimination throughout evolution, it is not surprising that human aggression appears to have a strong genetic underpinning (Maynard Smith et al. 1988).

It should be noted that two types of aggressive behaviour are frequently distinguished, one resulting from a lack and the other from an excess of emotional sensitivity. The former behaviour is often described as instrumental, or proactive, aggression and is pre-meditated and frequently goal-directed. It is characteristically associated with psychopathy, lacking both empathy and remorse. This is in contrast to, so called, reactive aggression which is typically triggered by negative experiences and emotions including anger and/or anxiety (e.g. Tremblay et al. 2005). It appears to result from exaggerated threat perception and response to it, together with an inability to control the resultant enhanced emotional state (e.g. see Blair 2004; Blair et al. 2006; Crick and Dodge 1996).

In respect of reactive aggression and the response to threat, there appears to be a complex relationship between anxiety and aggression and a strong evolutionary conservation of the brain regions implicated for both in mammals. The neural circuits involved centre on the amygdala and its linked pathways which control avoidant, defensive or aggressive behaviour (e.g. see Lesch 2005). Indeed, it has been posited that dysfunction of the neural circuits regulating emotion represent an aetiological factor of impulsive violence (Davidson et al. 2000). These circuits embrace not only the amygdala, but also the anterior cingulated cortex and regions of the prefrontal cortex. Some individuals who show impulsive, violent tendencies have dysfunctional serotonergic projection to this region, consistent with the long held belief that disruption of the serotonin system is a highly significant feature in predisposing aggression (see Siever 2008; Berman et al. 2009).

Heritability of aggression

A detailed evaluation and meta-analysis of 24 genetically informative studies concerning aggression concluded that heritability accounted overall for about 50% of the variance (Miles and Carey 1997; Rhee and Waldman 2002). Miles and Carey concluded that "heritability and common environment are definitely responsible for individual differences in aggression". They noted, however, that heritability changed with time; whilst genetic factors and common environment were equally important in childhood, heritability became even more prominent in adulthood. It also appeared that male heritability for the trait was slightly higher than that for females.

Genetic features and likely pathways implicated in aggression

Sex differences and aggression

As noted above, there are likely to be different evolutionary aggression strategies for males and females resulting in different proactive and reactive responses in level and frequency. In humans, very clear cut distinctions between the sexes can be made on the basis of crime statistics which indicate that males are overwhelmingly more likely to be implicated in crimes involving aggression. In the USA, government data indicate males to be ten times more likely than females to commit murder and more than five times as likely to be under 'correctional supervision' for criminal offences (see http://www.ojp.usdoj.gov/bjs/). This appears to be a general finding in other populations; for example, in New Zealand, a longitudinal study of about 1,000 individuals over the period from 3 to 21 years, males were found to be 2.4 times more likely to be involved in antisocial behaviour than females (Moffitt et al. 2001). The widespread and consistent pattern of male excess in aggression statistics is remarkable given that it generally emerges irrespective of the measures employed and the age of the individuals surveyed.

A wealth of studies has demonstrated a correlation between testosterone levels and aggression in animals. The situation with humans is much less clear and the data more conflicting. Whilst some research has suggested that aggression and testosterone are indeed positively correlated (Archer 1991), there are contrary observations. For instance, Turner (1994) showed that whilst testosterone was positively correlated with aggressive behaviour in 12–13-year-old boys, it was not in 15–16-year-old boys. Subsequently, a meta-analysis, based on 45 independent studies encompassing 9,760 individuals and reflecting a range of both negative and positive correlations provided support for an overall weak positive effect ($r = 0.14$) (Book et al. 2001). Interpretation of the relationship is, however, complicated in humans, not only because of methodological problems, but also because of fluctuations in hormone levels in response to environmental conditions and circadian rhythm. Age is also a confound both in regard to changing response and actual levels.

Many investigations have concentrated on the aggressive behaviour of adolescent and young adult males referred to by Wilson and Daly (1985) as the ''Young Male Syndrome". Indeed, it is males between the ages of 12 and 25 who are the principal perpetrators and victims of violence, and this may be due to increasing testosterone levels which start to rise in early adolescence (e.g. Mazur 1983). Puberty signals increasing sexual maturity, a concomitant rise in androgen levels, and an increase in inter-male competition—a combination with a presumptive selection for confrontational competitive abilities (Wilson and Daly 1985; Book et al. 2001). This male-associated aggression suggests the possibility that genes in androgen synthesis and function are potentially implicated.

It appears that not only is the Y chromosome important in contributing to aggression through its role in male determination, but that other Y-linked loci may be significant. By sophisticated manipulations, it has been possible to delete the normal male determining gene (*Sry*) from the Y chromosome in mice (designated Y^-) and to provide it as an autosomal copy against a background of different X, Y and Y^- sex chromosome combinations. Analysis of the behaviours of the strains created clearly shows an effect on aggression independent of the *Sry* locus (Gatewood et al. 2006). This implies that other specific genes on the X and or Y chromosome are involved in aggression. With regard to the Y chromosome, this provides a restricted search field for any genes implicated; however, apart from some evidence suggesting the

implication of the steroid sulphatase locus, *Sts* (which incidentally is non-functional in humans, and hence unlikely to contribute to genetic variability) no other Y-chromosome loci have been confirmed as contributing to aggression.

Stress and the HPA axis

It follows from the hypothesis that failure to regulate emotional balance can result in impulsive reactive aggression that the stress response itself and its genetic components are likely to be implicated (see Craig 2007a). The hypothalamus, pituitary and adrenal (HPA) axis, frequently monitored through cortisol levels, which are directly affected by stress, has consequently been a focus for attention. Research into potential association between cortisol levels and aggression have, however, been hampered by the sampling protocols employed and the complications presented by diurnal variation together with immediate contextual mediated changes. Furthermore, the pubertal stage in the young individuals surveyed is an important variable. In an attempt to surmount such difficulties and to establish a trait-like, as opposed to a state-like, measure, Shirtcliff et al. (2005) monitored individual differences in a large number (724) of youths concentrating on early morning saliva samples. For the males only, higher externalising problem behaviour was consistently associated with lower salivary cortisol. Other recent studies have provided some support for this pattern. Loney et al. (2006) found that male adolescents exhibiting elevated callous unemotional traits had low cortisol levels compared to equivalent controls, and Popma et al. (2007) found that adolescent boys, who had disruptive behaviour disorder, showed significantly lower levels in the first hour after waking. Most recently, radio-immune assays were employed to compare key components in the saliva of 20 aggressive and 20 non-aggressive students matched for gender, age and pubertal development. Interestingly, although the male aggressive students manifested lower cortisol levels, higher testosterone levels predicted aggression in the girls (but not the boys) examined (Yu and Shi 2009). There are deviations from this apparent consensus, for example, van Bokhoven et al. (2005) employing a longitudinal approach to behavioural monitoring found consistently higher cortisol levels in boys with conduct disorder (particularly reactive aggression) than those without; however, levels were only monitored at age 13, thereby restricting more general interpretations.

The serotonin pathway

A rich history of research indicating the key role of serotonin in aggression indicates that the genetic control of all aspects of serotonin metabolism and particularly of its synthesis, its release from neurons and its action via the various receptors represent a rich field for the selection of candidate genes. One strand of evidence for this is the generally consistent observation for both humans and macaques that reduced levels of the serotonin metabolite 5-hydroxy-indole acetic acid (5-HIAA) in cerebrospinal fluid (CSF) are associated with violent behaviour (e.g. Birger et al. 2003; Coccaro et al. 1997). There has also been a persisting documentation of an association of low levels of 5-HIAA in the spinal fluid of suicide attempters and specifically in those employing violent means (see Birger et al. 2003 for review).

The action of serotonin is mediated by a range of cognate receptors. In addition to the receptors on the postsynaptic neuron, the involvement of the 5-HT1A and 5-HT1B autoreceptors on the pre-synaptic neuron also provide a routes for the regulation of aggressive behaviour, and there is evidence for this role in rodents. For example, up-regulation of somatodendritic 5-HT1A and terminal 5-HT1B autoreceptors is observed in highly aggressive rats, which is further enhanced following victorious aggressive experiences (Caramaschi et al. 2007). Such up-regulation may be part of a normal compensatory mechanism to the elevated activity of the serotonin system in such aggressive animals. If this autoreceptor response overshoots, it is possible that the resultant damping of the serotonergic neurons' activity leads to violent and aggressive behaviour (Caramaschi et al. 2007).

In contrast to the apparent damping down effect of serotonin, Coccaro et al. (1998) found in studies on CSF arginine vasopressin (AVP) and 5-HIAA levels that whilst the former were positively correlated with life history of aggression, the latter were correlated inversely. It therefore seems that loci implicated in the synthesis of and response to AVP may represent a further category of genetic variables for consideration.

Aggression and low blood sugar levels

Abnormally low levels of blood sugar (hypoglycaemia) may trigger a series of physiological changes and behaviours including aggression. Two types of non-diabetic hypoglycaemia are recognised. The term "reactive hypoglycaemia" describes recurrent episodes of symptomatic hypoglycaemia occurring 2–4 h after a high carbohydrate meal (or oral glucose load, OGL). It is thought to represent a consequence of excessive and persisting levels of insulin release triggered by the intake of carbohydrate/glucose. The second type is "fasting hypoglycaemia" also called post-absorptive hypoglycaemia, which may be related to an underlying disease state. Other predictors include hormonal deficiencies, intake of certain medications and/or alcoholic beverages (particularly "binge drinking"). The symptoms associated with significant hypoglycaemia include fatigue, dizziness, headaches and irritability; hence, there have been

several investigations of the association between low blood sugar and aggression. Virkkunen and Huttunen (1982) tested offenders who had committed one or more serious assaults and noted that they also responded in the OGL challenge with atypically low glucose levels and slow recovery times. Subsequently, Virkkunen (1983, 1986) found that insulin secretion was enhanced (with presumptive glucose level depletion) during tests on atypical responders and was associated with pronounced antisocial characteristics. Benton (1988) concludes that moderate falls in blood glucose may predispose individuals to aggression, but because the levels are not clinically within the range for hypoglycaemia, other normally observed symptoms may not manifest, thereby muddying the clarity of a formal demonstration between reactive hypoglycaemia and aggression. It seems, therefore, that moderate falls in glucose levels, caused for whatever reason (but including excessive alcohol intake), may cause irritability and depending on the levels of provocation and aggression.

Various studies have attempted to link serotonin mechanisms, insulin levels and glucose metabolism with aggression and impulse control, leading to the proposal of a "low serotonin syndrome" (e.g. Linnoila and Virkkunen 1992). It is predicted that a decrease in serotonin activity in the Raphe nuclei (which can result from excessive alcohol intake) causes a chain reaction, first in suppressing pancreatic insulin release, resulting in hypoglycaemia with concomitant increases in impulsivity and aggressive or violent behaviour (Yamamoto et al. 1984). Unfortunately, although it is reasonable to assume that the interactions between serotonin, glucose and alcohol metabolism are significant features of aggression, these findings are not conclusive enough as yet to have predictive value (e.g. see Virkkunen et al. 2007).

Evidence for the implication of specific genes

In the following sections, the evidence for the involvement of specific genes is considered. Following a brief review of genome wide studies, the functional variation in candidate genes, selected on the basis of their likely involvement in relevant pathways (as described in the preceding sections), will be reviewed.

Genome scans for aggression loci

Surprisingly, there appears to have been very few linkage or association whole genome scans for aggression apart from a search for quantitative trait loci in mice (Brodkin et al. 2002). In this study, outcrosses and backcrosses between the extremely aggressive strain, NZB/B1NJ, and the markedly unaggressive A/J strain have enabled two significant chromosomal regions to be identified and potential candidate loci to be suggested (Brodkin et al. 2002). The regions implicated were distal chromosome 10 and proximal chromosome X, with corresponding putative loci designated *Aggr1* and *Aggr2*. Candidate genes within the regions include the diacylglycerol kinase subunit gene (*Dagk1*) and the glutamate receptor subunit AMPA3 gene (*Gria3*), respectively. Plausible arguments can be made for both as candidates. Diacylglycerol kinases are involved in the phosphatidylinositol signal transduction system important in brain neurotransmission. In rats, glutamate receptor genes impart different pharmacologic and kinetic properties on currents evoked by L-glutamate or its analogue, AMPA. Indeed, subsequent evidence has shown that a selective agonist of glutamate receptors 2 and 3 can reduce the isolation-induced aggression in male mice (Navarro et al. 2008). Nevertheless, there appears to be no direct evidence that either locus is implicated in human aggression.

Candidate genes related to sex differences

Androgen receptor (AR)

The best established, highly polymorphic and functional locus with regard to sex determination is the androgen receptor (*AR*), which embraces two trinucleotide repeats. In a preliminary finding which will require replication, Jonsson et al. (2001) observed tendencies (not reaching statistical significance levels) in healthy Swedish males for an association of shorter (and presumptively higher expressed) CAG repeats with muscular tension and with verbal aggression. More recently, Rajender et al. (2008) have attempted to replicate this observation in Indian males convicted for various aggression-related offences (murder and/or rape) together with controls. They found a highly significant association of the shorter CAG repeats motifs with the somewhat more dramatic phenotype, in this case, of violent criminal activity.

Candidate genes in the serotoninergic system

Monoamine oxidases (MAOA and MAOB)

MAOA and MAOB are two closely related enzymes, the products of two abutting X-linked genes which play an important role in the metabolism of biogenic amines in the central nervous system and in the periphery. In general, whilst MAOA preferentially oxidises biogenic amines such as serotonin (5-HT), norepinephrine (NE) and epinephrine, MAOB is important in dopamine metabolism and degrades dietary amines including phenylethylamine. Of the two, by far the most investigated with respect to human aggression is *MAOA*. An important watershed in the candidature of

this locus followed the correlation between null mutations (leading to imbalances in serotonin and NE metabolism) and aggression observed in both human and mice (Brunner et al. 1993; Cases et al. 1995). Subsequently, most interest has centred on detecting behavioural associations with SNPs, microsatellites and/or promoter VNTR variants in *MAOA*. The latter are known to confer significant functional variation and given the high LD across the locus, it is possible that the associations with aggression and violence observed with other markers result from their acting as surrogates for the tandem repeat (reviewed in Craig 2007a; D'Souza and Craig 2008). This VNTR polymorphism has established functional effects on reporter genes and expressed proteins. It is located 1.2 kb upstream of the coding region and comprises a 30-bp repeated sequence normally present in 3, 3.5, 4 or 5 copies. The 3 and 4 repeats are predominant and have been classified as low or high expression forms, respectively (Deckert et al. 1999; Denney et al. 1999; Sabol et al. 1998); however, one study has failed to detect associated significant transcriptional differences (Balciuniene et al. 2002) employing human cortical brain tissue as opposed to cultured cells. Interestingly, Pinsonneault et al. (2006), also working with human brain observed that other *cis* acting factors may obscure any differential transcriptional effect of the 4 versus the 3 repeat (see also Cirulli and Goldstein 2007). There are conflicting reports of a direct association of low activity alleles and a range of aggressive behaviours (see Craig 2005). The complex interactions between functional variation at this locus, exposure to environments and sex hormones that may be responsible for the inconsistencies are reviewed in a later section.

Serotonin transporter (SLC6A4)

Surprisingly, given the role of serotonin modulation and aggression, the well-established long and short versions of the promoter region (*5-HTTLPR*) encoding high and low activity variants have figured only slightly in context of a potential role in human aggression. Support is mainly provided by a study on violent and non-violent adult males referred for forensic assessment. The results interestingly showed an interaction effect between childhood high adversity impacting only on later-life violence if the short (low activity) promoter alleles were present (Reif et al. 2007).

Tryptophan hydroxylases 1 and 2 (TPH1 and TPH2)

The traditionally investigated tryptophan hydroxylase enzyme (TPH1) catalyses the rate-limiting step in the synthesis of serotonin and was thought to be widely expressed in the brain. Its candidature in behavioural studies was, however, somewhat complicated by the discovery in 2003 of a second form of the enzyme, TPH2 (Walther and Bader 2003). In mice, whilst *Tph2* is predominantly expressed in brain stem, *Tph1* is expressed in a wide range of tissues, but not predominantly in the brain. Some reports indicate that two human isoforms are both expressed in brain tissues (e.g. Zill et al. 2007); however, very recently Gutknecht et al. (2009) were unable to detect TPH1 by immuno-histochemistry or in situ hybridisation in adult human or mice brain, not in brain of developing mice.

TPH1 has two well-characterised SNPs (A218C and A779C) in intron 7, which are in strong linkage disequilibrium. The 779A allele (also referred to as U) has been associated with lower CSF 5-HIAA in control males (but not females); however, the lowest 5-HIAA levels were found in homozygous individuals for the C allele in impulsive alcoholic violent offenders (Nielsen et al. 1994). Manuck et al. (1999) also investigated the A218C polymorphism in a sample comprising 124 men and 127 women. Overall, individuals with an A allele scored significantly higher on measures of aggression and tendency to experience unprovoked anger. The co-variation of *TPH1* genotype with aggression and anger measures was found to be statistically robust in men, but non-significant amongst women. Hence, the overall conclusion from several studies suggest that the two SNPs (although given their high LD unlikely to be operating independently) are probably directly, or indirectly, implicated in both the regulation of CSF 5-HIAA levels and in aggression, as well as having a possible role in suicidal behaviour (see Hennig et al. 2005). The latter authors examined the association of the A779C SNP with aggression subdivided into aggressive hostility (AH) and neurotic hostility (NH)—corresponding roughly to "proactive" and "reactive" aggression. Similar to the observations of Manuck et al. (1999), individuals homozygous for the A allele showed the highest aggression and CC individuals the lowest; but only for AH and not NH. Hennig et al. conclude that the dichotomy observed for the subdivisions may explain some of the inconsistencies in the literature; however, the study was relatively small and requires replication.

There is an inter-strain polymorphism of the *Tph2* gene in mice. This C1473G variation encodes a Pro447 to Arg447 substitution and is associated with brain TPH activity and inter-male aggressiveness across ten mouse strains (Kulikov et al. 2005); a study extended and confirmed by Osipova et al. (2009). The Pro447Arg substitution corresponds to the Pro449Arg polymorphism in the human enzyme TPH2 (Zhang et al. 2004). Although this has been associated with affective disorders, there is surprisingly as yet no evidence presented for a role in aggression. Transmission disequilibrium analysis has, however, suggested a role for variants in the transcriptional control region of TPH2 in ADHD (Walitza et al. 2005).

Serotonin receptors

5-HT1A and 5-HT1B receptors associated with serotonergic neurons appear to be key players in the control of offensive aggression in rodents. 5-HT1B hetero-receptors (i.e. modulating neurotransmitter release on non-5-HT nerve terminals) have also been identified as important in modulating offensive aggression. Together with the serotonin transporter, control of 5-HT release, via presynaptic 5-HT1A and 5-HT1B, (auto) receptors may also have important influences on aggression under certain conditions (for review see Olivier and van Oorschot 2005). Several studies have sought to establish whether, or not, altered function of the 5-HT1B (hetero) receptor in humans may contribute to changes in aggressive behaviour. There are a number of polymorphisms in the coding sequence and surrounding 5′- and 3′-untranslated regions, and more than 20 association studies with aggression have been published with varying results (Sanders et al. 2002). A functional polymorphism, G861C, has been identified which affects binding of serotonin (Huang et al. 1999), and associations have been found with antisocial alcoholism (Hasegawa et al. 2002; Lappalainen et al. 1998; Soyka et al. 2004) and also with a history of suicide attempts (New et al. 2001); however, other investigations failed to link the polymorphism with suicidal behaviour or suicide (Nishiguchi et al. 2001; Rujescu et al. 2003; Stefulj et al. 2004), whilst yet other studies have reported associations with pervasive aggression in children (Davidge et al. 2004).

There are several polymorphisms reported also for *5HT2A*; however, there is scant evidence for the functional importance of any. Most studies have focused on a single SNP providing a silent T102C polymorphism (rs6313) and have provided support for association with a range of behaviours, including psychosis, agitation, aberrant motor behaviour and depression (e.g. see Prichard et al. 2008); however, these authors were unable to replicate a previous association reported for the CC genotype with agitation and aggression in Alzheimer's disease patients (Lam et al. 2004). A second well-studied polymorphism is the G-1438A promoter SNP which is in nearly complete linkage disequilibrium with the silent T102C SNP has been examined in context of antisocial behaviours in alcohol dependents, and has been found to have significant association with impulsive behaviour but not borderline or antisocial, personality disorders (Preuss et al. 2001).

Candidate genes in the stress response pathway

Two separate elements in the stress pathway exist, the autonomic reaction to stressful situations (commonly typified by the fight or flight paradigm) and the neuroendocrine stress response. The list of possible genetic factors interacting with these systems is open-ended; however, potential significant functional variation in a selection of the top candidate genes has been reviewed recently (Craig 2007b). It is worth noting, however, in spite of the key position occupied by the glucocorticoid receptor and the extensive variation reported for the *GR* locus including 16 SNPs (Bray and Cotton 2003), there have been no consistent observations linking these directly to aggressive behaviour.

Dopamine-beta-hydroxylase (DBH)

DBH is a key enzyme in the synthesis of norepinephrine, and there is an abundance of literature describing the genetic control of DBH levels (in serum) and some indication that this may underpin aspects of antisocial behaviour. In an early study, Rogeness et al. (1982) reported that children with conduct disorder (under-socialised type) had low plasma DBH levels. Subsequently, it was observed that males with early experience of maltreatment (and consequently potentially at risk for the subsequent development of aggressive traits) had relatively low DBH levels (Galvin et al. 1991); furthermore, similar low levels were observed in young boys with behavioural disorders and whose fathers showed antisocial behaviour (Gabel et al. 1995).

Several polymorphisms including a SNP (C-1021T, rs1611115), which accounts for about 50% variance in plasma levels, and a dinucleotide repeat 4 kb upstream have been employed in association studies. Most recently, Hess et al. (2009) have provided evidence that the *DBH*-1021TT genotype was significantly associated with increased neuroticism scores and impulsive and/or aggressive behaviour in ADHD.

Catechol-O-methyl transferase (COMT)

The COMT enzyme catalyses the transfer of a methyl group from *S*-adenosylmethionine to catecholamines, including dopamine and epinephrine; this provides a major degradative pathway for the catecholamine transmitters. Knock out of *COMT* in mice leads to increased aggressive behaviour but only in males (Gogos et al. 1998). There are, however, contradictory reports on the relationship between *COMT* and aggressive behaviour in humans. The studies have mainly concentrated on the functional SNP (val158met) in the coding region, with the met substitution leading to approximately 40% reduction in enzyme activity by conferring thermolability. Some authors have found a correlation of the met allele with increased aggressiveness particularly for males and frequently associated with schizophrenia. Recently, given the ambiguity of the data and the role of sex differences, Kulikova et al. (2008) examined the functional SNP in the manifestation of physical aggression in 114 unselected women. They observed that

the met/met homozygotes are least aggressive, whilst wild type homozygotes (val/val) exhibited maximum aggression ($P < 0.01$).

Adrenergic receptors

Evidence for a link between norepinephrine and aggressive behaviour comes from a variety of studies employing animal models. Such research has found in general that there is a positive relationship between noradrenergic activity and fighting/biting behaviour in various rodents and monkeys. In most human studies, a positive relationship between aggressiveness and CSF norepinephrine, or its metabolite 3-methoxy-4-hydroxyphenylglycol and MHPG are found (e.g. Brown et al. 1982; Placidi et al. 2001). It is therefore of some interest that the β-type noradrenergic receptor blocker (propranolol) has been employed to control aggressive behaviour in violent patients (for review see Yudofsky et al. 1998). Studies that have administered propranolol to a small number of violent patients have reported a reduction of aggressive behaviour in some but not all (Silver et al. 1999). This suggests that genetic variation in the NE receptors—particularly ADRB1 (on which beta blockers typically act) may be important in both stress and aggression responses. A functional SNP (arg389 allele) in *ADRB1* has been associated with changes to heart rate and BMI; however, a direct link between functional variation in the beta adrenergic receptors and aggression remains to be firmly established.

Noradrenaline transporter (NET1, SLC6A2)

Although alterations in the concentration of NE in the CNS have been reported with major psychiatric disorders, Robertson et al. (2001), who reviewed a variety of polymorphisms that had been described in the *NET* locus, concluded that there was no evidence that they contributed to any psychiatric illness. Subsequently, however, Kim et al. (2006) identified a −3081A/T polymorphism in the *NET* promoter with the T allele showing significantly decreased promoter function. They also presented preliminary evidence suggesting an association between the −3081T allele and ADHD in Americans of European descent, thereby providing a tenuous link with aggression.

Overall, whilst it seems that there is considerable theoretical and some experimental evidence suggesting the importance of the stress response pathways in modulating aggression, the ability to demonstrate direct and reproducible links between established functional variation in the major genes investigated and human aggression remains elusive.

Candidate genes related to hypoglycaemia

The physiological link between low blood glucose and aggression has yet to be explained adequately. Nevertheless, an interesting development is that variation in the serotonin transporter-linked polymorphic region (*5-HTTLPR*) was found to affect nutritional intervention on fasting blood glucose (FBG) levels in non-diabetic females with the low activity homozygotes showing significantly larger decreases in FBG over the test period than other genotypes (Yamakawa et al. 2005). This provides a tangential aspect to the role of genetic variation in the serotonin transporter in context of aggression. Furthermore, glucose transporters are also thought to have an affect on hypoglycaemia and resultant behaviour, and it can be posited that any decrease in transporter functionality could exacerbate hypoglycaemic symptoms, including an increase in irritability. This is supported by studies on a family, in whom six out of the eight family members with dystonia-18 were found to have a mutation of the glucose transporter *SLC2A1* gene, resulting in irritability and impulsive behaviour (Weber et al. 2008).

Other candidates

Nitric oxide synthase (NOS1)

Since the first observation that a knock out of *Nos1* in mice results in increased aggression (Nelson et al. 1995), there have been many animal studies which have supported a role for the enzyme in this and related behaviours. Very recently, Reif et al. (2009) have carried out an in-depth study of the structure of this complex gene and have concentrated their investigations on a functional promoter repeat length variant (*NOS1* Ex1f VNTR). This area contains recognition sites for a range of transcription factors. In a study of more than 3,200 individuals, association was found with traits relating to impulsivity, including hyperactive and aggressive behaviour. Interestingly, the short version of the repeat which was associated is also characterised by lowered transcription of the *NOS1* exon 1f promoter and alterations in the pattern of neuronal transcripts. The lower activity and associated behavioural consequences of the short VNTR allele is reminiscent of those observed for *MAOA* and *SERT*. We suggest that variation at the *NOS1* locus is likely to provide a valuable basis for future investigations.

Arginine vasopressin receptor (AVPR1A)

Given the evidence that the levels of arginine vasopressin have been implicated in aggression (Coccaro et al. 1998), it would seem likely that functional variation in the cognate

receptors might have a significant role. The receptor gene *AVPR1a* has promoter repeats, which in lower vertebrates are associated with regulation of brain expression patterns. Furthermore, *Avpr1a* has been implicated in aggressive behaviour in rodents (Ferris et al. 1997). In humans, two microsatellite repeats in the reporter have been shown to be associated with social communication and also autistic traits; however, as yet no direct relationship to aggression has been demonstrated (Bachner-Melman et al. 2005; Kim et al. 2002; Wassink et al. 2004).

Gene × environment interactions: the role of stress in aggression

The overwhelming conclusion from both linkage and candidate gene studies is that there are few, if any, loci with large effect size, and it is becoming increasingly obvious that it will be necessary to consider the impact of genes, not in isolation, but as part of a multifactorial miasma including both other genetic factors and the environment. Strong evidence that this is the case stems from the replicated observations of interactions between the functional variants in the *MAOA* locus and stressful upbringing. In the first study of this kind, it was found that maltreated males were significantly more likely to develop antisocial problems if they had the low activity genotype in the 5′ regulatory VNTR (Caspi et al. 2002). There have been several subsequent studies, the majority of which have confirmed the basic conclusion that the high activity promoter variant confers protection against stressful and abusive childhood (Foley et al. 2004; Nilsson et al. 2006; Widom and Brzustowicz. 2006). The generality of the observation has been confirmed by meta-analysis (Kim-Cohen et al. 2006) with a stronger interaction effect in males (Taylor and Kim-Cohen 2007). Others have found MAOA genotype and adverse childhood environments act independently of each other in increasing the risk for later-life violent behaviour (Reif et al. 2007).

A separate interpretation has been put forward by Huang et al. (2004) who found a significant correlation of the high expression MAOA-uVNTR polymorphism with lower impulsivity, but only in adult males who report early childhood abuse. They proposed from their observations that the polymorphism may be a marker for impulsivity and consequently provide an increased risk for abuse, which may lead on subsequently to aggressive behaviour. Interestingly, Nilsson et al. (2007) noted that high MAOA activity male individuals were less likely than their low activity counterparts to be involved in destructive behaviour during adolescent alcohol consumption. Most recently, Weder et al. (2009) have investigated the G × E interaction at a range of trauma levels, and whilst confirming a significant interaction between exposure to moderate trauma and the low-activity *MAOA* genotype for both males and females (hemi or homozygous, respectively) found that exposure to extreme levels of trauma resulted in high aggression scores regardless of genotype. Overall, it seems clear that this complex situation remains an area for continued study—with requirements for standardisation of phenotype and environmental assessments.

There are relatively few studies relating to *MAOA* functional variants and female behaviour; however, a G × E interaction has been observed in which girls with high, rather than low, activity alleles appeared to be at increased risk of committing criminal behaviour in the presence of psychosocial risk (Sjoberg et al. 2007). More recently, a main effect of low activity *MAOA* alleles on risk for conduct disorder in females but not in males was detected; but a significant interaction with adversity was not observed (Prom-Wormley et al. 2009).

Furthermore, with regard to the possible interaction with both sex and stress, the evidence that *MAOA* transcription may be regulated by both androgens and glucocorticoids (via the HPA axis) may provide a mechanistic insight (see Craig 2007a). Comprehensive studies show that activation of the *MAOA* promoter region by glucocorticoids and androgens is regulated differently by R1 (RAM2/CDCA7L) and Sp1 transcription factors. It has been demonstrated that androgens interact directly with the third of the glucocorticoid/androgen response elements (GRE/ARE), which also appears to act indirectly with Sp1 (Ou et al. 2006). Glucocorticoid activation of transcription at these sites is stronger than that observed for androgens and, as they compete for the same site; it is possible that high testosterone levels may lead to an overall lower expression of *MAOA* (Ou et al. 2006). This remains to be confirmed. Of additional interest in this context is the recent observation that levels of *MAOA* activity determined by the promoter VNTR appear to interact non-additively with testosterone in predicting antisocial behaviour (as measured by the Brown–Goodwin lifetime scale aggression score). Whilst high levels of testosterone are associated with increased scores, individuals with the high activity MAOA alleles behave similarly to those with lower testosterone levels. As the authors comment, this may explain in part the lack of consistency in attempts to correlate testosterone and violence (Sjoberg et al. 2008).

Brain imaging results support the involvement of MAOA

Meyer-Lindenberg et al. (2006) have shown that low activity *MAOA* genotype in healthy males appeared to predicate significant reductions in volume of virtually the entire

cingulated gyrus and bilateral amygdalae. There were also genotype-dependent differences in amygdala activation during emotional arousal. They observed a significant sex-specific genotype interaction with only males increasing the volume of their bilateral lateral orbitofrontal cortex (OFC) by 14% volume in low activity genotypes relative to high-activity genotypes; however, no genotype-dependent structural changes were present in this region in women. Blood oxygenation level-dependent (BOLD) fMRI studies showed that in response inhibition task, whilst a greater response in healthy males was observed in the Brodmann's area in high activity *MAOA* genotypes, in low activity genotypes a greater response was observed in the right superior parietal cortex and bilateral extrastriate cortex (Passamonti et al. 2006). In contrast to the general failure to detect main effects of the MAOA genotype in association studies, Eisenberger et al. (2007) reported a direct effect of genotype on aggression traits in their imaging cohort, with both sexes reporting higher trait aggression if they were low activity. They also showed greater dorsal anterior cingulate cortex (dACC) reactivity suggesting that the MAOA–aggression relationship was mediated by greater reactivity of this brain region to social exclusion.

In the first attempt to correlate brain MAOA activity measured by positron emission tomography with the labelled ligand, C^{11}chlorgyline, no significant differences were observed between the high- and low-activity genotypes, although a trend for higher activity was observed in the predicted direction for the visual cortex of high *MAOA* individuals (Fowler et al. 2007). In subsequent publications by the same group (Alia-Klein et al. 2008a, b, 2009), it was shown that lower brain activity in cortical and sub-cortical brain regions correlated with higher self reported trait aggression (observed in both high and low genotype groups); however, in a challenge which involved emphatically delivering the word *NO,* carriers of the low *MAOA* genotype differed in reactions of the brain regions involved in the control of anger, which may underlie their greater vulnerability to aggressive behaviour.

It seems, therefore, that genotype–brain and genotype–behaviour relationships are developmentally complex. Other detailed functional, structural and connectivity investigations have suggested that the low activity *MAOA* allele adversely prejudices information processing within the amygdala, rostral cingulate and medial prefrontal cortex. Reduced rates of metabolism of low activity carriers lead to altered serotonin and NE levels. If this occurs during a critical window for the development of corticolimbic circuitry, this may disrupt normal social and emotional adjustment leaving individuals more vulnerable to the influence of adverse early life experience (see Buckholtz and Meyer-Lindenberg 2008).

Postscript: the potential role of epigenetics

Following the intriguing observations concerning the methylation of the glucocorticoid receptor promoter in rat pups exposed to maternal deprivation and their subsequent behavioural deficits (Weaver et al. 2004), it has been shown that adult male rats which had been exposed to maternal deprivation showed increased inter-male aggressiveness (Veenema et al. 2007). The potential for similar molecular mechanisms in human aggression provides an intriguing area for subsequent research and it is of potential relevance that there is evidence for an apparently analogous methylation of the glucocorticoid receptor gene in humans as revealed by studies on violent suicide victims who had been exposed to early adverse life events (McGowan et al. 2009). Of specific interest in this context is the report of Pinsonneault et al. (2006), who demonstrated CpG methylation of the MAOA promoter region in female, but not male, brain tissue; however, the possible significance of this in context of G × E interactions remains to be elucidated.

It may be that in the absence of substantial and widely replicated main effects of the genes investigated to date, the future of research in the genetics of human aggression lies in the area of epigenetic modifications resulting from environmental interaction. This emphasises the need to document adequately a range of environmental variables, particularly centred on early trauma and persistent stress, for those individuals who will form the next cohorts for whole genome studies. There also remains the possibility that genome features (particularly those lying out of reach of current SNP based screening techniques) may have a major contribution to the aetiology of aggression. Such features may include rare copy number variants and complex tandem repeats. It is clear that there is an exciting range of new directions in which to pursue genome research on human aggression.

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Chapter 18

Drug Addictions and Genetics of the Dopamine Pathway

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Abbreviations

ADH Alcohol dehydrogenase
ALDH Aldehyde dehydrogenase
ANKK1 Ankyrin repeat and kinase domain containing 1
COMT Catechol-*O*-methyltransferase
DAT1 Dopamine active transport 1
DBH Dopamine beta-hydroxylase
DRD1 Dopamine receptor D1
DRD2 Dopamine receptor D2
DRD3 Dopamine receptor D3
DRD4 Dopamine receptor D4
DRD5 Dopamine receptor D5
GABA Gamma-aminobutyric acid
GABRA2 Gamma-aminobutyric acid A receptor, alpha 2 subunit
GWAS Genome-wide association studies
miR microRNA
NCAM1 Neural cell adhesion molecule 1
OPRM1 Opioid receptor Mu 1
SLC6A3 Solute carrier family 6, member 3
SNP Single nucleotide polymorphism
TTC2 Tetratricopeptide repeat domain 12
VNTR Variable number of tandem repeats

INTRODUCTION

Drug addiction refers to substance use disorder according to the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, 2013). It constitutes a broad phenotype, encompassing behavioral, cognitive, and biological substrates but also involving sociological and economic factors (Stevens, 2011). Familial aggregation study further suggests that addiction runs in families, with a genetic component (Nugent et al., 2014). Twin studies have been widely used to measure the impact of genetic factors in a multifactorial behavior in humans. Genetic variants are shared completely (100%) between monozygotic cotwins, while dizygotic twins shared only 50% of their genetic variants on average, like any siblings. Twin studies examine two sources of environmental variation: one that members of a twin pair have in common (shared environment), the other being specific to an individual (nonshared environment). By definition the correlation of shared environment in a twin pair is 100% and the correlation of nonshared environment is 0%. Based on these assumptions, twin studies estimate that the heritability of drug addiction ranges between 30% and 60%, depending on the phenotype and the population studied (Vink, van Beijsterveldt, Huppertz, Bartels, & Boomsma, 2015). It should be emphasized that both sporadic structural changes in DNA and epigenetic modification, when not inherited, are not captured in twin studies (Hall, Drgonova, Jain, & Uhl, 2013). Moreover, the evidence that a significant proportion of drug addiction is genetic does not inform on the particular genes involved in drug addiction, how these genes induce an effect, or how many genes could be involved. During the past decades, the first approach used to identify vulnerability genes was the examination of candidate genes, based on the plausibility that a small set of genes could contribute to the risk of addiction according to our knowledge of its biological mechanisms. The more recent development of genome-wide association studies (GWAS), examining the association of many genetic variants with the phenotype without a priori hypothesis, have led to new insights into the role of genetic variants in drug addiction.

About 10,000 papers came out from the PubMed/NCBI database with the key words "gene, substance use, dopamine." This chapter is therefore not an extensive review of linkage or association studies examining genes involved in the dopamine circuitry and their role in the development of drug addiction in humans, but rather a focus on the most relevant and more recent studies in the field. For a complete overview of genetics in addiction, we refer the reader to articles examining either a particular substance such as opiates (Reed, Butelman, Yuferov, Randesi, & Kreek, 2014), alcohol (Banerjee, 2014), cocaine (Kreek et al., 2012), or the more specific pharmacogenetics aspect of drug addiction (Jones & Comer, 2015).

THE DOPAMINE PATHWAY

The majority of addictive drugs are used to trigger pleasure or relieve distress. Dopamine is a major modulator of the balance between reward and aversion (antireward), and plays a key role in different components of drug addiction, for example, the reinforcing effects of addictive drugs or their aversive effects.

The expression of either high or low levels of dopamine D2 receptors (D2R) in the striatum has been shown to predict the

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reinforcing effect of a psychostimulant in nonabusing individuals. Low levels of D2R may contribute to psychostimulant abuse by favoring pleasant response (Volkow et al., 1999), while high D2R may protect from psychostimulant abuse by favoring aversive effects (Volkow et al., 2002).

This lower striatal D2R availability persists even during acute withdrawal (Martinez et al., 2005) or long term after alcohol detoxification (Heinz et al., 2004; Martinez et al., 2005). Whether this lower availability is the cause or the consequence of drug use, it could modulate the risk of relapse shortly after withdrawal. This last hypothesis is further supported by the inverse correlation of striatal D2R availability with alcohol craving and cue-induced activation of several brain regions in participants detoxified from alcohol (Heinz et al., 2004). However, this finding has not yet been replicated for other substances (Fehr et al., 2008).

CANDIDATE GENE STUDIES OF ADDICTION WITHIN THE DOPAMINE PATHWAY

DRD2 Gene

Several genes are involved in the neurobiological dopaminergic pathway, leading different research teams to assess candidate gene variants associated with this pathway. The most striking example is the single nucleotide polymorphism TaqIA (SNP rs1800497) of the D2R, *DRD2* gene. This variant has been considered as a vulnerability gene for alcoholism in more than 60 case–control studies involving about 20,000 participants, with conflicting results. We are aware of nine reviews and meta-analyses on this topic (Le Foll, Gallo, Le Strat, Lu, & Gorwood, 2009; Munafo, Johnstone, Welsh, & Walton, 2005; Noble, 1998; Smith, Watson, Gates, Ball, & Foxcroft, 2008), again with conflicting results. Table 1 reports the major results of these meta-analyses.

Interestingly, and in accordance with the role of dopamine in the detection of potential reward facets of external stimuli, the A1 allele carriers of the rs1800497 learned to avoid actions with negative consequences less efficiently (Klein et al., 2007). This finding has been confirmed by a paper examining both the impact of the administration of a selective dopamine D2/3-receptor antagonist and the role of the TaqIA variant on reinforcement learning (Eisenegger et al., 2014). In this work, the TaqIA variant modulates the effect of the D2/3-receptor antagonist in this reinforcement learning paradigm.

TABLE 1 Key Facts on the *DRD2* Gene and Addiction

- The TaqIA/rs1800497 variant of the D2R gene is a functional polymorphism.
- This variant has been studied in more than 100 association studies in addiction.
- Meta-analysis showed conflicting results.
- The association is positive for alcohol dependence.
- For other (nonalcohol) substances, a high heterogeneity between studies is observed.

Altogether, these data suggest that A1 allele carriers are less likely to learn from the negative experiences, including theoretically the negative consequences of a use, and thus may be more likely to develop alcohol addiction (Le Strat, Ramoz, Pickering, et al., 2008; Le Strat, Ramoz, Schumann, & Gorwood, 2008).

This indirect involvement could partly explain the discrepant results observed in previous association studies. Moreover, dense mapping of the *DRD2* locus suggests association with adjacent genes, namely, *ANKK1*, *TTC2*, and *NRCAM1* (Dick et al., 2007). TaqIA variant changes the amino acid in the *ANKK1* gene and might confer functional biological consequences in alcohol use disorder (Dubertret et al., 2004). The genomic organization of the locus *DRD2/ANKK1* includes several SNPs, including TaqIA (Figure 1).

OTHER DOPAMINE-RELATED GENES AND ADDICTION

Catechol-*O*-methyltransferase (COMT) metabolizes dopamine (Table 2). The human *COMT* gene contains a functional polymorphism (Val158Met, SNP rs4680), with individuals carrying the Val allele having a 40% higher enzyme activity than Met homozygotes, at least in the frontal cortex (Chen et al., 2004). COMT Val158Met variant modulates prefrontal activity, particularly working memory and executive function (Egan et al., 2001). The involvement of COMT in reward processing is support by a growing literature. For a more in-depth discussion, we refer the reader to a review by Tunbridge et al. (2012). For example, COMT Val158Met allele has an impact on the striatal activation during a reward anticipation (Yacubian et al., 2007), and on the ability to discriminate between gains and losses (Marco-Pallares et al., 2009). Similarly, in a delay discounting task, participants carrying the Val allele were more likely

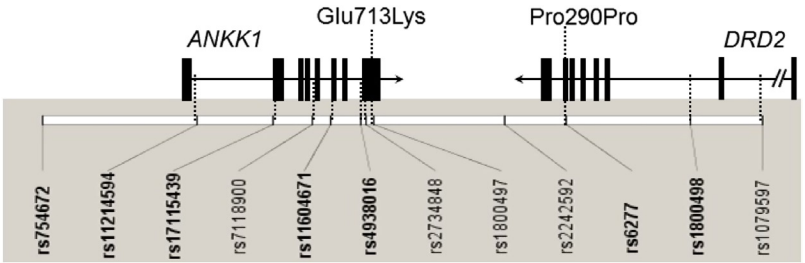

FIGURE 1 Genomic organization of the *DRD2/ANKK1* locus. From bottom to top of the figure, list of selected SNPs across the *DRD2/ANKK1* locus; vertical bars and boxes indicate the exons of the *DRD2* and *ANKK1* genes; arrows represent the orientation of the transcription of the genes; names of the genes are indicated in italic; the correspondence of the amino acids for the rs1800497 and rs6277 is noted, respectively, Glu713Lys and Pro290Pro. SNPs, Single nucleotide polymorphisms.

TABLE 2 Key Facts on the *COMT* Gene and Addiction

- COMT metabolizes dopamine.
- The Val158Met variant is functional, the Val allele having a higher enzyme activity.
- COMT modulates cortical and striatal activation during reward anticipation and delivery.
- Meta-analyses of association studies considering alcohol or illicit drugs are negative.
- COMT influences drug addiction-related phenotypes.

to choose a smaller but immediate reward rather than a delayed but larger reward (Boettiger et al., 2007; Tunbridge et al., 2012).

COMT appears to modulate cortical and striatal activation during reward anticipation and delivery, and to modulate reward-based learning; COMT Val158Met polymorphism is therefore a good candidate for association in the field of addiction (Tunbridge et al., 2012). However, association studies between COMT and drug addiction-related phenotypes have produced mixed results, meta-analyses considering alcohol or illicit drugs being negative. However, studies were conducted in relatively small samples, with a high heterogeneity. Studies examining the association of the Val158Met polymorphism with smoking-related behavior found some modest but significant association, further confirmed by meta-analysis (Munafo, Freathy, Ring, St Pourcain, & Smith, 2011; Tammimaki & Mannisto, 2010).

It is likely that COMT influences drug addiction-related phenotypes. For example, work has focused on neonatal abstinence syndrome (NAS), a disorder associating various symptoms including neurologic, gastrointestinal, and respiratory signs because of in utero opiates exposure. In this work, COMT Val158Met variant was associated with NAS itself, but also with length of hospital stay and need for more intensive treatment in infants with NAS (Wachman et al., 2013).

Other dopamine receptor genes have been linked with drug addiction. For a complete review of this area, we refer the reader to Gorwood et al. (2012). Briefly, 31 SNPs have been identified in the *DRD1* gene, and 10 association studies have been conducted to date on different drug addictions (alcohol use disorder, heavy smoking, and methamphetamine use) most of them being negative (Gorwood et al., 2012). A total of 508 SNPs have been identified in the *DRD3* gene, again most of them being negative except from an association with nicotine dependence that was detected in preliminary studies, but warrants further replications (Gorwood et al., 2012). The *DRD5* gene is one exon that encodes a protein of 477aa. Among the 300 SNPs, rs7655090 was associated with a withdrawal severity score in alcohol dependence (Hack et al., 2011). Otherwise, no association was observed between *DRD5* SNPs and addictions (Gorwood et al., 2012). The role of a variable number of tandem repeats (VNTR) polymorphism located in the third exon of the *DRD4* gene is among the most convincing (Gorwood et al., 2012). While the association with alcohol use disorder is heterogeneous, its role in nicotine dependence has been more consistent (Le Foll et al., 2009). Moreover, a meta-analysis confirmed the association of the L allele of this polymorphism with opioid dependence risk (Le Foll et al., 2009).

Another gene of the dopaminergic pathway is *SLC6A3* (solute carrier family 6, member 3)/*DAT1* (dopamine active transport 1), which codes for the dopamine reuptake transporter. Alcohol-dependent patients who were carriers of the allele of nine repeats of the VNTR located in the 15th exon in the 3′ noncoding region of *DAT1* were associated with alcohol withdrawal complications (Le Strat, Ramoz, Pickering, et al., 2008; Le Strat, Ramoz, Schumann, et al., 2008). This gene has also been investigated in the pharmacogenetics of treatment response in alcohol dependence. An epistasis effect between the VNTR of *DAT1* and the Asp40 allele of the *OPRM1* gene, which encodes the opioid receptor Mu 1, was therefore published for the clinical response to naltrexone in alcohol-dependent patients (Anton, Voronin, Randall, Myrick, & Tiffany, 2012).

DOPAMINE IN GENOME-WIDE ASSOCIATION STUDIES ON ADDICTION

GWAS allows the possibility to analyze hundreds of thousands of SNPs in the absence of a priori assumption. Four GWAS on alcohol dependence, for example, have been published so far. They have shown limited replicability, but support the role of the gene *GABRA2*, which encodes the GABA receptor 2 subunit (Gorwood et al., 2012) and they confirm the association of genes involved in the metabolism of alcohol (alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH)) (Samochowiec, Samochowiec, Puls, Bienkowski, & Schott, 2014). However, no GWAS to date provided significant association of genetic variants on dopamine receptor genes, including the *DRD2* gene. Finally, the meta-analysis of GWAS on alcohol dependence also failed to find SNPs among dopamine receptor genes in the top 25 SNPs significantly associated with this addiction (Wang et al., 2011).

Interestingly, whole genome studies have been successfully applied in tobacco dependence. One SNP, rs3025343, which is located upstream of the gene encoding the dopamine B hydroxylase (the *DBH* gene, the enzyme that converts the dopamine to noradrenaline) was associated with smoking cessation (Thorgeirsson et al., 2010; Tobacco and genetics consortium, 2010).

DOPAMINE AND EPIGENETICS IN ADDICTION

The rs6277 SNP of the *DRD2* gene (Figure 1) was associated with alcohol dependence. This SNP is in linkage disequilibrium with rs1800497 and corresponds to the synonymous mutation of the amino acid proline 290. The presence of this mutation, and other synonymous mutations among the *DRD2* gene, may affect the stability of the gene transcript, leading to a decreased microRNA (miR) stability and translation, therefore reducing the synthesis of the DRD2 receptor (Duan et al., 2003).

In fact, inherited genetic polymorphisms are not the only sources of transcript variance. Genetic expression is indeed under the control of promoters and enhancers that can be variable according to their genetic polymorphisms, but also under the control of epigenetic effects, including methylation of the DNA, histones modifications of chromatin, and regulations by miRs. Interestingly epigenetic mechanisms are involved in the regulation of the

TABLE 3 Published Meta-Analysis of *DRD2* TaqIA Variant and Drug Addictions

First Author	Year of Publication	Phenotype Studied	Number of Studies Included	Main Result
Deng et al.	2015	Illicit drug dependence	25	Positive for opioid dependence negative for stimulants or marijuana dependence
Wang et al.	2015	Alcohol dependence	61	Positive
Chen et al.	2014	Opioid dependence	22	Positive
Le Foll et al.	2009	Alcohol dependence	40	Positive
Munafo et al.	2009	Smoking	29	Negative
Smith et al.	2009	Alcohol dependence	44	Positive
Munafo et al.	2007	Alcohol dependence	40	Positive
Munafo et al.	2005	Social drinking	2	Positive
Munafo et al.	2004	Smoking	13	Negative for smoking initiation Negative for persistent smoking

Note that most of the meta-analyses published so far are positive. However, the effect size is generally low.

genome to preserve it under environmental changes. Novel technics in molecular biology are now able to measure such epigenetic effects. Two independent studies, for example, reported that the hypermethylation of the promoter of the *DAT1* gene was negatively correlated with alcohol craving (Hillemacher et al., 2009; Nieratschker et al., 2014).

Studies devoted to miRs in alcohol dependence performed up to now, either from postmortem brain or from blood samples, reported some miRs that in fact target genes involved in neuroinflammatory processes (Lewohl et al., 2011; Nunez & Mayfield, 2012). Nevertheless, it has been shown that D2R expression in the brain is regulated by miR-9 and miR-326 (Shi et al., 2014). Furthermore, the SNP rs1130354, located in the 3′ untranslated region of the *DRD2* gene, interferes with miR-326 to modify its impact of *DRD2* expression (Shi et al., 2014). Their role in alcohol dependence and other addictions is therefore of major interest (Table 3).

CONCLUSION

Recent studies have confirmed the role of dopamine-related genes in addictive disorders. The existing literature supports an association of dopamine genes with a higher risk of addiction through different components of drug addiction, including the reinforcing or aversive effects of drugs, but also the ability to learn through reward processing, or to discriminate between gains and losses. The preference for immediate reward rather than higher, but delayed, reward has been well described in patients with drug addiction. The direct role of dopamine genes on the development of drug addiction or through these different features is not clear yet.

Most recent GWAS have not confirmed the identification of dopamine genes. Concluding on such negative results requires taking into account the nonspecific, large, and heterogeneous phenotypes used in such studies. Indeed, GWAS usually rely on permissive inclusion criteria to facilitate the recruitment of very large samples, a prerequisite for this approach. Focusing on a transdiagnostic mechanism (e.g., a reward-related system) rather than on the broad addiction phenotype constitutes avenues for further research. The development of molecular biology tools is also promising in allowing investigation of the epigenetic mechanisms that could be involved in addiction. They could open novel areas of investigations regarding the diagnosis, prognosis, and understanding of the physiopathology of addiction.

APPLICATION TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

1. Studies of dopamine genes give new insight into the physiopathology of drug addictions and substance misuse.
2. Most of the genes involved (e.g., *DRD2*, *COMT*) play a role on the reward process.
3. These genes are likely to play a role in drug addiction in general rather than in a specific substance of abuse (Figure 2).
4. No GWAS has provided significant association of a genetic variant on dopamine receptor genes and a single substance use disorder.
5. Future study should examine the role of dopamine genes with more restricted phenotypes associated with addiction.
6. Understanding the epigenetics in addiction will allow for the identification of biomarkers and pathophysiological pathway linking genes and environmental events.

DEFINITION OF TERMS

Candidate gene The candidate gene approach directly tests the effect of genetic variants of a selected gene on a phenotype. The choice of the suitable gene is critical, as it should be relevant for the physiopathology of the disorder under investigation.

Dopamine genes Genes within the dopamine signaling pathway. Dopamine-related genes encode for enzymes, receptors, or transporters of dopamine.

Epigenetics Mechanisms that change the expression of the genes in regard to environmental events without modification of the sequence of the gene.

GWAS GWAS is an abbreviation for genome-wide association studies, also known as whole genome association studies, using microarray technology to identify association between a phenotype and a genomic region.

SNP SNP (pronounced "snip") is an abbreviation for single nucleotide polymorphism, the most common genetic variation, in which a single nucleotide differs between individuals. If more than 1% of a population does not carry the same allele at a locus, this variation is called a SNP.

Variable number tandem repeat (VNTR) A VNTR is a repeated unit that is 10–100 nucleotides long.

SUMMARY POINTS

- This chapter focuses on the genetics of dopamine in drug addiction.
- The heritability of drug addiction ranges between 30% and 60%.
- Dopamine is a major modulator of the balance between reward and aversion.
- Levels of D2R in the striatum play a central role in drug addiction.
- Low levels of D2R favor pleasant response to a drug of abuse.
- High D2R density may safeguard against drug addiction.
- The genetic variant TaqIA/rs1800497 of the D2R *DRD2* gene is the most studied.
- Other variants, including COMT Val158Met polymorphism, play an important role.

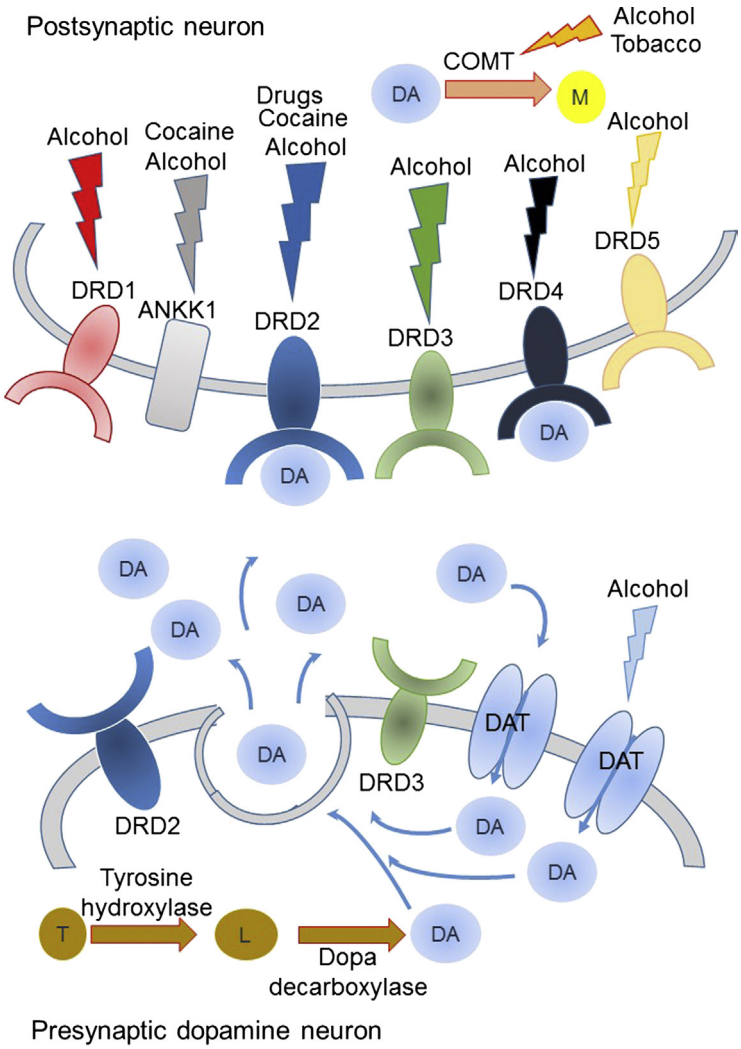

FIGURE 2 Schematic summarizing of genetic associations of the dopamine pathway with substance dependences. In the dopamine neurons, there is a production of dopamine (DA) after the hydroxylation of the tyrosine (T) amino acid by the tyrosine hydroxylase and the decarboxylation of the L-Dopa (L). The dopamine neurotransmitter is secreted by the presynaptic dopaminergic neuron. At the postsynaptic neuron are the dopamine receptors 1–5 (DRD1–DRD5). Then, the dopamine is methylated to 3-methoxytyramine metabolite (M) by the catechol-*O*-methyltransferase enzyme (COMT). Furthermore, at the membrane of the presynaptic dopaminergic neuron, there are the dopamine receptors 2 and 3 (DRD2 and DRD3) and, the dopamine transporter (DAT), which is able to reuptake the dopamine from the synaptic cleft. The positive genetic associations between a variant of a gene encoding one of the factors of the dopamine pathway and a dependence to a substance are mentioned according to the lightning bolt. TTC2, Tetratricopeptide repeat domain 12. *From a review by Gorwood et al. (2012).*

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The Genetics of Alcohol and Other Drug Dependence

Danielle M. Dick, Ph.D., and Arpana Agrawal, Ph.D.

Alcohol dependence and dependence on other drugs frequently co-occur, and strong evidence suggests that both disorders are, at least in part, influenced by genetic factors. Indeed, studies using twins suggest that the overlap between dependence on alcohol and on other drugs largely results from shared genetic factors. This common genetic liability, which also extends to antisocial behavior, has been conceptualized as a general predisposition toward a variety of forms of psychopathology characterized by disinhibited behavior (i.e., externalizing psychopathology). Accordingly, many of the genetic factors affecting risk for dependence on alcohol or other drugs appear to act through a general externalizing factor; however, other genetic factors appear to be specific to a certain disorder. In recent years, researchers have identified numerous genes as affecting risk for dependence on alcohol and other drugs. These include genes involved in alcohol metabolism as well as in the transmission of nerve cell signals and modulation of nerve cell activity (i.e., γ-aminobutyric acid [GABA] and acetylcholinergic neurotransmission and the endogenous opioid and cannabinoid systems). *Key words: Alcohol and other drug (AOD) dependence (AODD); co-morbid AOD dependence; genetics and heredity; genetic theory of AODD; genetic risk factors; AODR genetic markers*

This article explores the hypothesis that certain genetic factors increase a person's risk of both alcohol abuse and dependence and other drug abuse and dependence. It first reviews the evidence suggesting that certain genetic factors contribute to the development of alcohol and other drug (AOD) use disorders, as well as to the development of a variety of forms of externalizing psychopathology—that is, psychiatric disorders characterized by disinhibited behavior, such as antisocial personality disorder, attention deficit/hyperactivity disorder, and conduct disorder. After summarizing the difficulties associated with, and recent progress made in, the identification of specific genes associated with AOD dependence, the article then discusses evidence that implicates several genes in a person's risk for dependence on both alcohol and illicit drugs.

Genetic Epidemiology of AOD Dependence

Alcohol dependence frequently co-occurs with dependence on illicit drugs (Hasin et al. 2007). Both alcohol use disorders (i.e., alcohol abuse and alcohol dependence) and drug use disorders (drug abuse and drug dependence) are influenced by several factors. For example, family, twin, and adoption studies[1] have convincingly demonstrated that genes contribute to the development of alcohol dependence, with heritability estimates ranging from 50 to 60 percent for both men and women (McGue 1999). Dependence on illicit drugs only more recently has been investigated in twin samples, but several studies now suggest that illicit drug abuse and dependence also are under significant genetic influence. In these studies of adult samples, heritability estimates ranged from 45 to 79 percent (for reviews, see Agrawal and Lynskey 2006; Kendler et al. 2003*a*; Tsuang et al. 2001).

Twin studies also can be used to assess the extent to which the *co-occurrence* of disorders is influenced by genetic and/or environmental factors. Thus, a finding that the correlation between alcohol dependence in twin 1 and drug dependence in twin 2 is higher for identical (i.e., monozygotic) twins, who share 100

[1] For a definition of these and other terms, see the glossary, pp. 177–179.

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percent of their genes, than for fraternal (i.e., dizygotic) twins, who share on average only 50 percent of their genes, indicates that shared genes influence the risk of both alcohol and drug dependence. The twin studies conducted to date support the role of such shared genetic factors. For example, in the largest twin study of the factors underlying psychiatric disorders, Kendler and colleagues (2003*b*) analyzed data from the Virginia Twin Registry and found that a common genetic factor contributed to the total variance in alcohol dependence, illicit drug abuse and dependence, conduct disorder, and adult antisocial behavior. This pattern also has been identified in several other independent twin studies (Krueger et al. 2002; Young et al. 2000). Taken together, these findings suggest that a significant portion of the genetic influence on alcohol dependence and drug dependence is through a general predisposition toward externalizing disorders, which may manifest in different ways (e.g., different forms of AOD dependence and/or antisocial behavior) (see figure). However, some evidence also suggests that disorder-specific genetic influences contribute to AOD dependence (Kendler et al. 2003*b*). These specific influences likely reflect the actions of genes that are involved in the metabolism of individual drugs.

The idea that alcohol and drug dependence share a genetic liability with each other, as well as with other forms of externalizing psychopathology, is further supported by electrophysiological studies recording the brain's electrical activity. These studies, which are conducted using electrodes placed on the person's scalp, provide a noninvasive, sensitive method of measuring brain function in humans. They generate a predictable pattern in the height (i.e., amplitude) and rate (i.e., frequency) of brain waves that can show characteristic abnormalities in people with certain types of brain dysfunction. For example, electrophysiological abnormalities have been observed in people with a variety of externalizing disorders as well as in unaffected children of these people. These findings suggest that electrophysiological measurements can be used as markers of a genetic vulnerability to externalizing disorders.

One commonly measured electrophysiological characteristic is the so-called P3 component of an event-related potential—that is, a spike in brain activity that occurs about 300 milliseconds after a person is exposed to a sudden stimulus (e.g., a sound or light). Researchers have observed that the amplitude of the P3 component is reduced in alcohol-dependent people and their children, suggesting that this abnormality is a marker for a genetic predisposition to alcohol dependence (Porjesz et al. 1995). However, the abnormal P3 response is not specific to alcohol dependence but appears to be associated with a variety of disinhibitory disorders, including other forms of drug dependence, childhood externalizing disorders, and adult antisocial personality disorder, again suggesting a shared underlying predisposition to multiple forms of AOD dependence and other externalizing problems (Hicks et al. 2007).[2]

Interestingly, electrophysiological abnormalities are most pronounced in alcohol-dependent people who also have a diagnosis of illicit drug abuse or dependence (Malone et al. 2001). This observation is consistent with data from twin and family studies suggesting that co-morbid dependence on alcohol and another drug represents a more severe disorder with higher heritability than dependence

[2] Abnormalities in the P3 response also have been associated with risk for other psychiatric disorders, such as schizophrenia (van der Stelt et al. 2004).

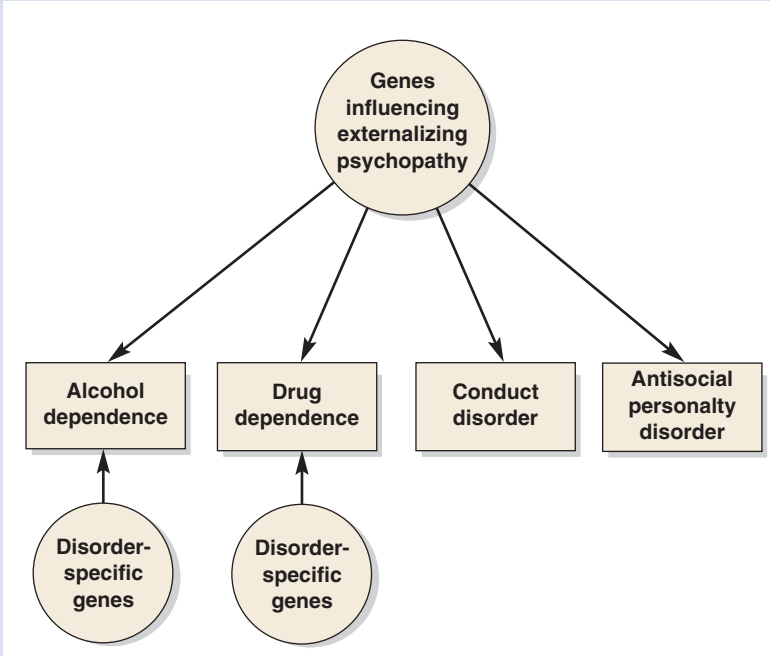

Figure Schematic representation of a model to illustrate the influence of genetic factors on the development of alcohol dependence, dependence on other drugs, and other externalizing disorders (e.g., conduct disorder or antisocial personality disorder). Some of the proposed genetic factors are thought to have a general influence on all types of externalizing conditions, whereas others are thought to have a disorder-specific influence.

on one drug alone (Johnson et al. 1996; Pickens et al. 1995). This conclusion also appears to be supported by new studies exploring the roles of specific genes, which are discussed later in this article.

IDENTIFYING SPECIFIC GENES RELATED TO AOD DEPENDENCE

With robust evidence indicating that genes influence both alcohol dependence and dependence on illicit drugs, efforts now are underway to identify specific genes involved in the development of these disorders. This identification, however, is complicated by many factors. For example, numerous genes are thought to contribute to a person's susceptibility to alcohol and/or drug dependence, and affected people may carry different combinations of those genes. Additionally, environmental influences have an impact on substance use, as does gene–environment interaction (Heath et al. 2002). Finally, the manifestation of AOD dependence varies greatly among affected people, for example, with respect to age of onset of problems, types of symptoms exhibited (i.e., symptomatic profile), substance use history, and presence of co-morbid disorders.

Despite the complications mentioned above, the rapid growth in research technologies for gene identification in recent years has led to a concomitant increase in exciting results. After suffering many disappointments in early attempts to identify genes involved in complex behavioral outcomes (i.e., phenotypes), researchers now are frequently succeeding in identifying genes that help determine a variety of clinical phenotypes. These advances have been made possible by several factors. First, advances in technologies to identify a person's genetic makeup (i.e., genotyping technology) have dramatically lowered the cost of genotyping, allowing for high-throughput analyses of the entire genome. Second, the completion of several large-scale research endeavors, such as the Human Genome Project, the International HapMap Project,[3] and other government and privately funded efforts, have made a wealth of information on variations in the human genome publicly available. Third, these developments have been complemented by advances in the statistical analysis of genetic data.

Several large collaborative projects that strive to identify genes involved in AOD dependence currently are underway. The first large-scale project aimed at identifying genes contributing to alcohol dependence was the National Institute on Alcohol Abuse and Alcoholism (NIAAA)-sponsored Collaborative Study on the Genetics of Alcoholism (COGA), which was initiated in 1989. This study, which involves collaboration of investigators at several sites in the United States, examines families with several alcohol-dependent members who were recruited from treatment centers across the United States. This study has been joined by several other gene identification studies focusing on families affected with alcohol dependence, including the following:

- A sample of Southwestern American Indians (Long et al. 1998);
- The Irish Affected Sib Pair Study of Alcohol Dependence (Prescott et al. 2005*a*);
- A population of Mission Indians (Ehlers et al. 2004);
- A sample of densely affected families collected in the Pittsburgh area (Hill et al. 2004); and
- An ongoing data collection from alcohol-dependent individuals in Australia.

Importantly, most of these projects include comprehensive psychiatric interviews that focus not only on alcohol use and alcohol use disorders but which also allow researchers to collect information about other drug use and dependence. This comprehensive approach permits researchers to address questions about the nature of genetic influences on AOD dependence, as discussed below.

More recently, additional studies have been initiated that specifically seek to identify genes contributing to various forms of illicit drug dependence as well as general drug use problems (for more information, see http://www.nida.nih.gov/about/organization/Genetics/consortium/index.html). Through these combined approaches, researchers should be able to identify both genes with drug-specific effects and genes with more general effects on drug use. The following sections focus on several groups of genes that have been identified by these research efforts and which have been implicated in affecting risk for dependence on both alcohol and illicit drugs.

Genes Encoding Proteins Involved in Alcohol Metabolism

The genes that have been associated with alcohol dependence most consistently are those encoding the enzymes that metabolize alcohol (chemically known as ethanol). The main pathway of alcohol metabolism involves two steps. In the first step, ethanol is converted into the toxic intermediate acetaldehyde; this step is mediated by the alcohol dehydrogenase (ADH) enzymes. In a second step, the acetaldehyde is further broken down into acetate and water by the actions of aldehyde dehydrogenase (ALDH) enzymes. The genes that encode the ADH and ALDH enzymes exist in several variants (i.e., alleles) that are characterized by variations (i.e., polymorphisms) in the sequence of the DNA building blocks. One important group of ADH enzymes are the ADH class I isozymes ADH1A, ADH1B, and ADH1C. For both

[3] The International HapMap Project is a multicountry effort to identify and catalog genetic similarities and differences in human beings by comparing the genetic sequences of different individuals in order to identify chromosomal regions where genetic variants are shared. Using the information obtained in the HapMap Project, researchers will be able to find genes that affect health, disease, and individual responses to medications and environmental factors.

the genes encoding ADH1B and those encoding ADH1C, several alleles resulting in altered proteins have been identified, and the proteins encoded by some of these alleles exhibit particularly high enzymatic activity in laboratory experiments (i.e., in vitro) (Edenberg 2007). This suggests that in people carrying these alleles, ethanol is more rapidly converted to acetaldehyde.[4] Several studies have reported lower frequencies of both the *ADH1B*2* and *ADH1C*1* alleles, which encode some of the more active proteins, among alcoholics than among nonalcoholics in a variety of East Asian populations (e.g., Shen et al. 1997) and, more recently, in European populations (Neumark et al. 1998; Whitfield et al. 1998).

In addition, genome-wide screens to identify genes linked to alcoholism and alcohol-related traits have been conducted in three independent samples consisting largely of people of European descent—the COGA study (Saccone et al. 2000), the Irish Affected Sib Pair Study of Alcohol Dependence (Prescott et al. 2005*a*), and an Australian sample (Birley et al. 2005). These studies have found evidence that a region on chromosome 4 containing the ADH gene cluster shows linkage to the phenotypes studied. This cluster contains, in addition to the genes encoding ADH class I isozymes, the genes *ADH4*, *ADH5*, *ADH6*, and *ADH7*, which encode other ADH enzymes. Polymorphisms exist for each of these genes, some of which also have been associated with alcohol dependence (Edenberg et al. 2006; Luo et al. 2006*a*,*b*; Prescott et al. 2005*b*).

Interestingly, the effects of these genes do not appear to be limited to alcohol dependence. One study compared the frequency of alleles that differed in only one DNA building block (i.e., single nucleotide polymorphisms [SNPs]) throughout the genome between people with histories of illicit drug use and/or dependence and unrelated control participants. This study detected a significant difference for a SNP located near the ADH gene cluster (Uhl et al. 2001). More recent evidence suggests that genetic variants in the *ADH1A*, *ADH1B*, *ADH1C*, *ADH5*, *ADH6*, and *ADH7* genes are associated with illicit drug dependence and that this association is not purely attributable to co-morbid alcohol dependence (Luo et al. 2007). The mechanism by which these genes may affect risk for illicit drug dependence is not entirely clear. However, other observations[5] also indicate that enzymes involved in alcohol metabolism may contribute to illicit drug dependence via pathways that currently are unknown but independent of alcohol metabolism (Luo et al. 2007).

Genes Encoding Proteins Involved in Neurotransmission

AODs exert their behavioral effects in part by altering the transmission of signals among nerve cells (i.e., neurons) in the brain. This transmission is mediated by chemical messengers (i.e., neurotransmitters) that are released by the signal-emitting neuron and bind to specific proteins (i.e., receptors) on the signal-receiving neuron. AODs influence the activities of several neurotransmitter systems, including those involving the neurotransmitters γ-aminobutyric acid (GABA), dopamine, and acetylcholine, as well as naturally produced compounds that structurally resemble opioids and cannabinoids. Accordingly, certain genes encoding components of these neurotransmitter systems may contribute to the risk of both alcohol dependence and illicit drug dependence.

Genes Encoding the $GABA_A$ Receptor. GABA is the major inhibitory neurotransmitter in the human central nervous system—that is, it affects neurons in a way that reduces their activity. Several lines of evidence suggest that GABA is involved in many of the behavioral effects of alcohol, including motor incoordination, anxiety reduction (i.e., anxiolysis), sedation, withdrawal signs, and preference for alcohol (Grobin et al. 1998). GABA interacts with several receptors, and much of the research on alcohol's interactions with the GABA system has focused on the $GABA_A$ receptor. This receptor also is the site of action for several medications that frequently are misused and have high addictive potential, such as benzodiazepines, barbiturates, opiates, α-hydroxybutyrates, and other sedative–hypnotic compounds. Accordingly, this receptor likely is involved in dependence on these drugs as well (Orser 2006).

The $GABA_A$ receptor is composed of five subunits that are encoded by numerous genes, most of which are located in clusters. Thus, chromosome 4 contains a cluster comprising the genes *GABRA2*, *GABRA4*, *GABRB1*, and *GABRG1*; chromosome 5 contains *GABRA1*, *GABRA6*, *GABRB2*, and *GABRG2*; and chromosome 15 contains *GABRA5*, *GABRB3*, and *GABRG3* (see http:// www.ncbi.nlm.nih.gov/sites/entrez?db=gene).

Interest in the $GABA_A$ receptor genes on chromosome 4 grew when this region consistently was identified in genome-wide scans looking for linkage with alcohol dependence (Long et al. 1998; Williams et al. 1999). Subsequently, COGA investigators systematically evaluated short DNA segments of known location (i.e., genetic markers) that were situated in the $GABA_A$ receptor gene cluster on chromosome 4. These studies found that a significant association existed between multiple SNPs in the *GABRA2* gene and alcohol dependence (Edenberg et al. 2004). This association has been replicated in multiple independent samples (Covault et al. 2004; Fehr et al. 2006; Lappalainen et al. 2005; Soyka 2007). In addition, the same SNPs in the *GABRA2* gene have been shown to be associated with drug dependence in both adults and adolescents (Dick et

[4] Rapid acetaldehyde production can lead to acetaldehyde accumulation in the body, which results in highly unpleasant effects, such as nausea, flushing, and rapid heartbeat, that may deter people from drinking more alcohol.

[5] For example, the medication disulfiram, which inhibits another enzyme involved in alcohol metabolism called aldehyde dehydrogenase 2 (ALDH2) and is used for treatment of alcoholism, has demonstrated a treatment effect in cocaine dependence (Luo et al. 2007).

al. 2006*a*), as well as with the use of multiple drugs in another independent sample (Drgon et al. 2006).

Variations in the *GABRA2* gene are associated not only with AOD dependence but also with certain electrophysiological characteristics (i.e., endophenotypes) in the COGA sample (Edenberg et al. 2004). As reviewed above, these electrophysiological characteristics are not unique to alcohol dependence but also are found in individuals with other forms of externalizing psychopathology. This association supports the hypothesis that the *GABRA2* gene generally is involved in AOD use and/or externalizing problems. Interestingly, subsequent analyses investigating the role of *GABRA2* in drug dependence (Agrawal et al. 2006) found that the association with *GABRA2* was strongest in people with co-morbid AOD dependence, with no evidence of association in people who were only alcohol dependent. This observation supports the assertion that co-morbid AOD dependence may represent a more severe, genetically influenced form of the disorder.

Several other $GABA_A$ receptor genes have yielded more modest evidence of association with different aspects of AOD dependence. Thus, *GABRB3* (Noble et al. 1998) and *GABRG3* (Dick et al. 2004) are modestly associated with alcohol dependence, *GABRA1* (Dick et al. 2006*b*) is associated with alcohol-related phenotypes (e.g., history of alcohol-induced blackouts and age at first drunkenness), and *GABRG2* (Loh et al. 2007) is associated with aspects of drug dependence. These findings await confirmation in independent samples.

Genes Involved in the Cholinergic System. The cholinergic system includes neurons that either release the neurotransmitter acetylcholine or respond to it. Acetylcholine generally has excitatory effects in the human central nervous system—that is, it affects neurons in a way that enhances their activity. It is thought to be involved in such processes as arousal, reward, learning, and short-term memory. One of the receptors through which acetylcholine acts is encoded by a gene called *CHRM2*. In the COGA sample, linkage was observed between a region on chromosome 7 that contains the *CHRM2* gene and alcohol dependence, and subsequent experiments confirmed that an association existed between alcohol dependence and the *CHRM2* gene (Wang et al. 2004). This association has been replicated in a large independent study (Luo et al. 2005) that also found evidence that the gene was associated with drug dependence.

As with the *GABRA2* gene described above, the association between *CHRM2* and alcohol dependence in the COGA sample was strongest in people who had co-morbid AOD dependence (Dick et al. 2007). Additional analyses in the COGA sample have suggested that *CHRM2* is associated with a generally increased risk of externalizing disorders, including symptoms of alcohol dependence and drug dependence (Dick et al. 2008). This potential role of *CHRM2* in contributing to the general liability of AOD use and externalizing disorders is further supported by findings that *CHRM2*, like *GABRA2*, also is associated with certain electrophysiological endophenotypes (Jones et al. 2004).

Genes Involved in the Endogenous Opioid System. Endogenous opioids are small molecules naturally produced in the body that have similar effects as the opiates (e.g., morphine and heroin) and which, among other functions, modulate the actions of other neurotransmitters. The endogenous opioid system has been implicated in contributing to the reinforcing effects of several drugs of abuse, including alcohol, opiates, and cocaine. This is supported by the finding that the medication naltrexone, which prevents the normal actions of endogenous opioids (i.e., is an opioid antagonist), is useful in the treatment of alcohol dependence and can reduce the number of drinking days, amount of alcohol consumed, and risk of relapse.

Research on the role of the endogenous opioids in AOD dependence has centered mainly on a gene called *OPRM1*, which encodes one type of opioid receptor (i.e., the μ-opioid receptor), although the results so far have been equivocal. This gene contains a polymorphism resulting in a different protein product (i.e., a nonsynonymous polymorphism) that in one study was found to bind one of the endogenous opioids (i.e., β-endorphin) three times as strongly as the main variant of the gene (Bond et al. 1998); other studies, however, could not confirm this finding (Befort et al. 2001; Beyer et al. 2004).

Laboratory studies have suggested that *OPRM1* is associated with sensitivity to the effects of alcohol (Ray and Hutchison 2004). In addition, several studies have reported evidence of an association between *OPRM1* and drug dependence (e.g., Bart et al. 2005). Other studies, however, have failed to find such an association (e.g., Bergen et al. 1997), and a combined analysis of several studies (i.e., a meta-analysis) concluded that no association exists between the most commonly studied *OPRM1* polymorphism and drug dependence (Arias et al. 2006). However, this finding does not preclude the possibility that other genetic variants in *OPRM1* and/or other genes related to the endogenous opioid system are involved in risk for drug dependence. For example, a recent study determining the genotypes of multiple genetic variants across the gene uncovered evidence of association with *OPRM1* and AOD dependence (Zhang et al. 2006).

Researchers also have investigated genetic variations in other opioid receptors and other components of the endogenous opioid system; however, the results have been mixed. One study (Zhang et al. 2007) found modest support that the genes *OPRK1* and *OPRD1*—which encode the κ- and δ-opioid receptors, respectively—are associated with some aspects of drug dependence. Other researchers (Xuei et al. 2007) reported evidence that the genes *PDYN*, *PENK*, and *POMC*—which encode small molecules (i.e., peptides) that also bind to opi-

oid receptors—may be associated with various aspects of drug dependence.

Genes Involved in the Endogenous Cannabinoid System. Endogenous cannabinoids are compounds naturally produced in the body that have a similar structure to the psychoactive compounds found in the cannabis plant and which bind cannabinoid receptors. The endogenous cannabinoid system is thought to regulate brain circuits using the neurotransmitter dopamine, which likely helps mediate the rewarding experiences associated with addictive substances. The main cannabinoid receptor in the brain is called CB1 and is encoded by the *CNR1* gene, which is located on chromosome 6. This gene is an excellent candidate gene for being associated with AOD dependence because the receptor encoded by this gene is crucial for generating the rewarding effects of the compound responsible for the psychoactive effects associated with cannabis use (i.e., Δ9-tetrahydrocannabinol). However, the findings regarding the association between *CNR1* and AOD dependence to date have been equivocal, with some studies producing positive results (e.g., Zhang et al. 2004) and others producing negative results (e.g., Herman et al. 2006). Most recently, Hopfer and colleagues (2006) found that a SNP in the *CNR1* gene was associated with cannabis dependence symptoms.[6] Moreover, this SNP was part of several sets of multiple alleles that are transmitted jointly (i.e., haplotypes), some of which are associated with developing fewer dependence symptoms, whereas others are associated with an increased risk for cannabis dependence. Finally, a recent case–control study found that multiple genetic variants in *CNR1* were significantly associated with alcohol dependence and/or drug dependence (Zuo et al. 2007).

[6] The SNP was not located in one of those gene regions that encode the actual receptor (i.e., in an exon) but in a region that is part of the gene but is eliminated during the process of converting the genetic information into a protein product (i.e., in an intron).

Conclusions

For both alcohol dependence and drug dependence, considerable evidence suggests that genetic factors influence the risk of these disorders, with heritability estimates of 50 percent and higher. Moreover, twin studies and studies of electrophysiological characteristics indicate that the risk of developing AOD dependence, as well as other disinhibitory disorders (e.g., antisocial behavior), is determined at least in part by shared genetic factors. These observations suggest that some of a person's liability for AOD dependence will result from a general externalizing factor and some will result from genetic factors that are more disorder specific.

Several genes have been identified that confer risk to AOD dependence. Some of these genes—such as *GABRA2* and *CHRM2*— apparently act through a general externalizing phenotype. For other genes that appear to confer risk of AOD dependence—such as genes involved in alcohol metabolism and in the endogenous opioid and cannabinoid systems—however, the pathways through which they affect risk remain to be elucidated. Most of the genes reviewed in this article originally were found to be associated with alcohol dependence and only subsequently was their association with risk for dependence on other illicit drugs discovered as well. Furthermore, studies that primarily aim to identify genes involved in dependence on certain types of drugs may identify different variants affecting risk, underscoring the challenge of understanding genetic susceptibility to different classes of drugs.

This review does not exhaustively cover all genes that to date have been implicated in alcohol and illicit drug dependence. For example, several genes encoding receptors for the neurotransmitter dopamine have been suggested to determine at least in part a person's susceptibility to various forms of drug dependence. In particular, the *DRD2* gene has been associated with alcohol dependence (Blum et al. 1990) and, more broadly, with various forms of addiction (Blum et al. 1996). This association remains controversial, however, and more recent studies suggest that the observed association actually may not involve variants in the *DRD2* gene but variants in a neighboring gene called *ANKK1* (Dick et al. 2007*b*). Studies to identify candidate genes that influence dependence on illicit drugs, but not on alcohol, are particularly challenging because of the high co-morbidity between alcohol dependence and dependence on illicit drugs. Therefore, meaningful studies require large sample sizes to include enough drug-dependent people with no prior history of alcohol dependence.

The increasingly rapid pace of genetic discovery also has resulted in the identification of several genes encoding other types of proteins that appear to be associated with alcohol use and/or dependence. These include, for example, two genes encoding taste receptors (i.e., the *TAS2R16* gene [Hinrichs et al. 2006] and the *TAS2R38* gene [Wang et al. 2007]) and a human gene labeled *ZNF699* (Riley et al. 2006) that is related to a gene previously identified in the fruit fly *Drosophila* as contributing to the development of tolerance to alcohol in the flies. Future research will be necessary to elucidate the pathways by which these genes influence alcohol dependence and/or whether they are more broadly involved in other forms of drug dependence. ■

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