NEUROBIOLOGICAL CORRELATES OF AGGRESSIVE AND DISRUPTIVE BEHAVIOR IN CLINICALLY-REFERRED BOYS

by

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GLOSSARY OF ABBREVIATED TERMS

[^{11}C]MTrp - α-[^{11}C]methyl-L-tryptophan
[^{123}I]β-CIT - [^{123}I]2β-carbomethoxy-3β-(4-iodophenyl)-tropane
5-HT - 5-hydroxytryptamine or serotonin
5-HIAA - 5-hydroxyindoleacetic acid
8-OH-DPAT - 8-hydroxy-2-(di-*n*-prpylamino)
ADHD - attention-deficit/hyperactivity disorder
APD - antisocial personality disorder
CAS - Children's Aggression Scale
CBCL - Child Behavior Checklist
CD - conduct disorder
CHADD - Children and Adults with Attention Deficit Disorder
DBD - disruptive behavior disorders
DISC - Diagnostic Interview Schedule for Children
DOI - 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane
DSM-III-R - Diagnostic and Statistical Manual of Mental Disorders - Third Edition - Revised
DSM-IV - Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition
CSF - cerebrospinal fluid
FDG - [^{18}F]fluorodeoxyglucose
IOWA - IOWA Conners Teachers Questionnaire
MAO - monoamine oxidase
m-CPP - meta-chlorophenylpiperazine
ODD - oppositional defiant disorder
PET - positron emission tomography
PRF - prolactin releasing factor
PVN - paraventricular nucleus
SPECT - single photon emission computerized tomography
SSRI - selective serotonin reuptake inhibitors
TPH - tryptophan hydroxylase
VNTR - variable number tandem repeat
WISC-R - Wechsler Intelligence Scale for Children - Revised
WISC-III - Wechsler Intelligence Scale for Children - Third Edition

SPECIFIC AIMS

The mechanisms mediating the emergence of aggressive behavior in young children are not fully known, but involve a variety of psychosocial and environmental variables, as well as neurobiological factors. Specifically, considerable research has established a clear relationship between diminished central nervous system activity of the neurotransmitter serotonin (5-HT) and aggressive behavior in animals (reviewed in Soubrié, 1986) and human adults (reviewed in Zubieta & Alessi, 1993). In contrast, the few studies conducted in children have yielded variable results (Castellanos et al., 1994; Halperin et al., 1994, 1997a; Kruesi et al., 1990, 1992; Pine et al., 1997; Stoff et al., 1992). These results clearly indicate that the association between aggression and central 5-HT function in children is not characterized by the simple inverse relationship found in adults. Aggressive children are neither uniformly "high" nor uniformly "low" with regard to central 5-HT function (Halperin et al., 1997a,b). As such, the important question in children may not be "Do aggressive children have low (or high) 5-HT function relative to non-aggressive children?" Rather, the key question might be "What is the significance of low (or high) central 5-HT function in aggressive children?"

The heterogeneous nature of aggression in children might account for the inconsistency of central 5-HT findings in children. Only a small proportion of aggressive boys progress to serious violence as adolescents and adults (Loeber et al., 1993). Many factors influence whether or not a boy progresses towards violence (reviewed in Farrington & Loeber, 2000), and may obscure the role that low central 5-HT activity plays in this escalation. Yet, little is known regarding the extent to which low central 5-

HT function is associated with these variables, in particular familial aggression. Some evidence suggests that low central 5-HT function is associated with familial aggression and sociopathy (Coccaro et al., 1994; Halperin et al., 1997b).

Attempts by several of these investigators to reconcile the differences across the child studies of central 5-HT function and aggression noted that the samples differed in age and the proportion of subjects with attention-deficit hyperactivity disorder (ADHD) (Castellanos et al., 1994; Halperin et al., 1997a; Pine et al., 1997). These reviews raised the possibility that the association between central 5-HT and aggression varies as a function of age or ADHD. Specifically, central 5-HT activity: a) was greater in young aggressive boys relative to young nonaggressive boys, but did not differ among older boys (Halperin et al., 1997a); and b) was positively correlated with aggression in samples of boys with ADHD (Castellanos et al., 1994; Pine et al., 1997), but inversely correlated in samples that contained fewer cases of ADHD (Kruesi et al., 1990, 1992).

Seasonal fluctuations in 5-HT activity might also account for some of the variability among the child studies of central 5-HT activity. Seasonal variations in 5-HT activity have been described in adults, such that 5-HT is elevated in Autumn and reduced in Spring (Cappiello et al., 1996; Maes et al., 1995). However, evidence of seasonal variations in 5-HT activity in children is scant, and suggests that 5-HT seasonality in children varies as a function of psychiatric status (Pine et al., 1995). Group-specific seasonal differences in 5-HT could account for the variability and overlap between aggressive and nonaggressive boys in the child studies of central 5-HT.

This dissertation consists of a series of three studies that attempt to clarify the relationship between central 5-HT activity and aggressive and disruptive behavior in

clinically-referred boys. The primary goal of these studies is to assess the impact that age, the presence of ADHD, familial aggression and sociopathy, and seasonal fluctuations in 5-HT indices have on the relationship between central 5-HT and childhood aggression. Central 5-HT function was assessed in these studies by measuring the plasma prolactin response to a single oral dose of the 5-HT releaser/reuptake inhibitor *d,l*-fenfluramine (Cowen et al., 1990; Fuller , 1992). It was hypothesized that aggressive boys have reduced central 5-HT function as compared to nonaggressive boys, and consequently would exhibit lower prolactin responses to fenfluramine. However, this inverse relationship between central 5-HT and aggression would be masked by seasonal fluctuations in 5-HT indices or by age, the presence of ADHD, or familial aggression.

Specifically, it was hypothesized that:

1. Aggressive boys exhibit either a larger summertime and autumnal peak or a smaller springtime trough in central 5-HT activity than nonaggressive boys,
2. Central 5-HT activity is lowest in aggressive boys without ADHD and highest in aggressive boys with ADHD, with nonaggressive boys with ADHD falling in between,
3. Central 5-HT activity is greater in young aggressive boys relative to young nonaggressive boys, but does not differ among older boys,
4. Aggressive boys with reduced 5-HT function have an higher frequency of relatives with aggressive and antisocial characteristics than both nonaggressive boys and aggressive boys with higher 5-HT activity

INTRODUCTION

Recent violent incidents in schools across the country have once again focused public attention on the problem of juvenile crime and violence. The enormity of this problem is difficult to grasp, but the level of juvenile violence is reported to be much greater in the United States than in most other industrialized nations (Rosenberg, 1991). Moreover, the rate of juvenile violent crime has continued to rise over the past decade, despite a general decline in crime rates across the country during the same period (Snyder & Sickmund, 1995). Statistics such as these have prompted authorities across the country to adopt a "zero tolerance" approach to juvenile crime and enact tougher legislation to deal with the problem. Yet, experience has shown that legal measures alone can not adequately address the problem of juvenile violence. The increase in juvenile violence has also created an overwhelming demand for psychiatric and social services. Almost half of the juvenile referrals to community mental health centers and approximately one quarter of special services in school are for disruptive, delinquent, and aggressive behavior (Stouthamer-Loeber et al., 1992). Alarmingly, these children are at high risk for criminality, substance abuse, and antisocial behavior in adolescence and adulthood (Farrington, 1995). Scientific study of early childhood aggression and its antecedents is clearly required to deal with the problem of juvenile violence. The exploration of potential risk factors may lead to the early identification of children at high risk for a violent outcome and could pinpoint new directions for intervention strategies.

DEVELOPMENT OF CHILDHOOD AGGRESSION

Aggressive behavior is among the most stable of all early detectible personality characteristics (Loeber, 1991; Olweus, 1979). In fact, intelligence (i.e., IQ) may be the only trait that demonstrates greater stability across the life span (Olweus, 1979). However, the high stability of aggression is primarily accounted for by the most and the least aggressive children, and does not accurately reflect the considerable individual discontinuity in aggression over time (Loeber & Stouthamer-Loeber, 1998). Substantial change in the prevalence and manifestation of aggressive behavior has been observed across the life span (Loeber & Hay, 1997).

Physical aggression is most prevalent in early childhood and decreases subsequently throughout middle childhood and adolescence (Cairns et al., 1989; Loeber, 1982). Yet, at the same time, there is a concomitant temporal escalation in the severity of aggression. Many boys exhibit temper tantrums as infants and toddlers and engage in some minor aggression (e.g., bullying, annoying others) during the preschool and early elementary school years (Loeber & Hay, 1997). In fact, some aggression is developmentally-normative and not uncommon in preschool and young school-age boys (Loeber et al., 1997). However, there is a group of boys whose aggression is clearly not normative. As the majority of boys desist in their aggressive behavior during early to middle childhood (Loeber et al., 1989), a small proportion of aggressive boys persist in their maladaptive behavior and progress to serious physical fighting by early adolescence (Loeber et al., 1997). Moreover, an even smaller number of these aggressive boys escalate to greater violence (e.g., robbery, gang assault) during adolescence (Loeber et

Figure 1. Incidence of Murder in 1999 by Age of Offender. Based on data from the Federal Bureau of Investigation (FBI) Uniform Crime Report.

al., 1997). Consequently, as shown in Figure 1, the prevalence of serious violence (i.e., nonfamilial murder) increases during early adolescence, peaks during late adolescence and early adulthood, and then decreases with age (Loeber & Farrington, 1998), such that most forms of physically aggressive behavior are relatively rare among individuals beyond the age of 35 years.

The development of physically aggressive behavior in boys seems to progress over time through a series of stages at different ages (Loeber et al., 1993, 1997). The first stage of this developmental trajectory is characterized by minor aggressive behaviors such as annoying others and bullying, which usually emerge in the preschool and early school years. The second stage is marked by physical fighting and gang fighting, which accelerate from the age of 10 years onward. The third stage of the trajectory, which usually emerges during early adolescence, is characterized by serious violence, including

attacking someone and forcing sex. A substantial number of boys present with minor aggression, but only a small proportion progress to fighting and gang fighting, and even a smaller subgroup escalates to serious violence. Yet, few boys engage in serious violence without a history of physical fighting and, in most cases, minor aggression (Loeber et al., 1993, 1997).

A separate developmental trajectory has been proposed to explain a persistent pattern of covert antisocial behavior (Loeber et al., 1993, 1997). This trajectory begins with a sequence of minor covert behaviors (e.g., shoplifting, lying), followed by property damage (e.g., arson, vandalism), and finally escalating to serious criminal activity (e.g., fraud, burglary, theft). The separate developmental trajectories for overtly aggressive and covertly antisocial behavior attempt to account for the developmental differences of these largely overlapping behaviors.

The developmental progression of aggressive behavior in boys may seem clear, but the specific mechanisms that mediate this progression are still poorly understood. Individual, familial, peer, neighborhood, and neurobiological factors have all been linked to the emergence and development of aggressive behavior in boys. Moreover, these risk factors seem to operate in an additive fashion, with the probability of aggression increasing linearly with the aggregation of risk factors (Farrington, 1997; Rutter et al., 1975).

Individual Factors. A host of cognitive and personality variables have been linked to the emergence of aggressive behavior in boys, including low intelligence and specifically low verbal intelligence (Farrington, 1989), poor academic achievement (Hinshaw, 1992), social cognitive deficiencies (Dodge et al., 1984), inflated self-esteem

(Baumeister et al., 1996), emotional lability and poor impulse control (White et al., 1994), and oppositional behavior (Loeber et al., 1995). However, among the most salient risk factors for aggression in boys is the presence of attention-deficit hyperactivity disorder (ADHD) (Hinshaw, 1987; Sanson et al., 1993). The role of ADHD in the emergence of aggressive behavior appears to be several-fold. First, impulsivity is a core feature of ADHD. Second, ADHD is correlated with poor cognitive and academic functioning, as well as with the maintenance of oppositional behavior, all of which are also risk factors for aggression. Third, the presence of ADHD is associated with an early and accelerated development of aggressive behavior, conduct problems, and substance use in boys.

Familial Factors. High rates of criminality, aggressive behavior, antisocial personality disorder (APD), and alcohol and substance abuse have been found in the families of aggressive boys (Frick et al., 1992; Lahey et al., 1988). Aggressive children also tend to have greater family adversity, characterized by single parent households (Gagnon et al., 1995), child neglect and maltreatment (Smith & Thornberry, 1995), parental conflict (McCord, 1979), separated families, large numbers of siblings (Capaldi & Patterson, 1996), and family poverty (Lahey et al., 1988). Moreover, such parental child-rearing practices as physical discipline and poor supervision have been related to the emergence of childhood aggression (Frick et al., 1992). However, parental criminality or sociopathy may be the single most salient familial risk factor for the emergence and development of aggression in boys (Cadoret et al., 1995; Frick et al., 1992).

Peer Factors. Rejection by peers in early childhood and repeated victimization by

peers (e.g., target of bullying) in middle childhood are both predictive of an aggressive and violent outcome (Tremblay et al., 1988). Having delinquent friends becomes an important risk factor for a violent outcome as aggressive boys mature into adolescence (Cairns et al., 1988). Moreover, gang membership is associated with a significant increase in violent behavior that does not abate until membership ends (Thornberry et al., 1993).

Neighborhood Factors. Boys living in urban areas tend to be more aggressive and violent than their peers in rural locales (Elliot et al., 1989). More specifically, residing in a neighborhood with high rates of crime, poverty, unemployment, and/or female-headed households is associated with an early age of onset and accelerated progression of aggressive and violent behavior (Farrington & Loeber, 2000). The availability of guns and drugs in a neighborhood and the pervasiveness of gangs are also highly salient risk factors for youth violence (Blumenstein, 1995).

Neurobiological Factors. A host of neurobiological factors have been linked to the development of aggressive behavior in boys. Family, adoption, and twin studies have consistently demonstrated a substantial genetic contribution to the etiology of aggression (Cadoret et al., 1995; Coccaro et al., 1997a; Lahey et al., 1988). Reduced glucose metabolism in the orbitofrontal and adjacent medial frontal cortex has been reported in aggressive adults, and may be linked to emotional lability and deficits in impulse control (Critchley et al., 2000; Raine et al., 1997). Reduced autonomic nervous system reactivity suggestive of underarousal predicts criminal and aggressive outcome in boys (Raine et al., 1990). However, the most consistently reported neurobiological risk factor for aggressive behavior may be diminished central nervous system activity of the

neurotransmitter serotonin (5-HT) (reviewed in Zubieta & Alessi, 1993).

Compelling evidence indicates that the expression of aggressive behavior in boys is due to an interaction between biological and environmental risk factors (Cadoret et al., 1995). As such, identification of biological risk factors during early childhood may be an important step toward early intervention. This dissertation will focus on the role that diminished central 5-HT activity may play in the emergence of aggressive behavior in prepubertal boys. Over two decades of research has established an inverse relationship between central 5-HT activity and aggressive behavior in animals and human adults (reviewed in Soubrié, 1986; Zubieta & Alessi, 1993).

BASIC PROPERTIES OF CENTRAL 5-HT SYSTEM

The central 5-HT neurotransmitter system is relatively primitive in a number of characteristics (Jacobs & Fornal, 1999). The basic arrangement of 5-HT cell bodies in the brain is conserved throughout the vertebrate series, implying some commonality in physiology and function across fish, amphibians, reptiles, and mammals, including nonhuman primates and humans (Jacobs & Azmitia, 1992).

Anatomy. As shown in Figure 2, the cell bodies of the majority of 5-HT neurons in the brain lie near the midline of the brainstem in several clusters known collectively as the raphe nuclei (areas B1 through B9) (Dahlström & Fuxe, 1964). The raphe nuclei can be divided into two major groups (Jacobs & Azmitia, 1992). The caudal or inferior group consists of the nuclei raphe pallidus, raphe obscuris, and raphe magnus in the medulla (areas B1- B3), and the nucleus raphe pontis in the pons (areas B4 - B6), which

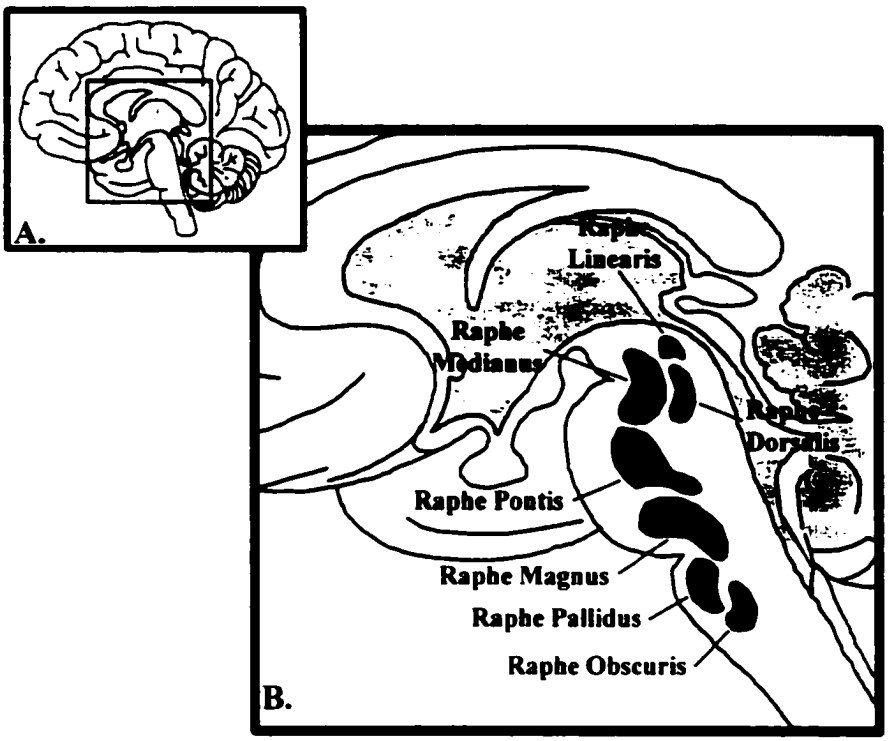

Figure 2. Anatomy of the Serotonin (5-HT) System in the Brain. A. Mid-sagittal view of the brain with area of detail highlighted. B. Detailed view of the brainstem with the raphe nuclei illustrated.

provide 5-HT innervation to the reticular formation and the spinal cord. The rostral or superior group comprises the midbrain nuclei raphe medianus, linearis, and dorsalis (areas B7 - B9), which provide 5-HT innervation to the diencephalon, the striatum, the septum, the amygdala, the frontal lobe, and other neocortical regions through three ascending pathways.

Synthesis and Metabolism. The brain synthesizes 5-HT from the precursor tryptophan , an essential amino acid that is provided by dietary sources (reviewed in Cooper et al., 1991). Plasma tryptophan competes with large neutral amino acids for active transport into the brain, where it is taken up by neurons. As shown in Figure 3,

tryptophan is then hydroxylated by the enzyme tryptophan hydroxylase to form 5-hydroxytryptophan, which is subsequently decarboxylated to 5-hydroxtryptamine or serotonin (5-HT) by the enzyme L-aromatic amino acid decarboxylase. Tryptophan hydroxylase is the rate-limiting step that determines 5-HT concentration in the brain. Synaptic 5-HT is inactivated by transport back into the presynaptic terminal, where it is metabolized by the enzymes monamine oxidase (MAO) and aldehyde dehydrogenase into 5-hydroxyindoleacetic acid (5-HIAA).

Receptors. The postsynaptic effects of 5-HT are transduced by fourteen different receptor proteins that are grouped into seven distinct families, 5-HT_1 through 5-HT_7 (Hoyer et al., 1994) (see Table 1). The 5-HT_1 receptor family includes the 5-HT_{1A}, 5-

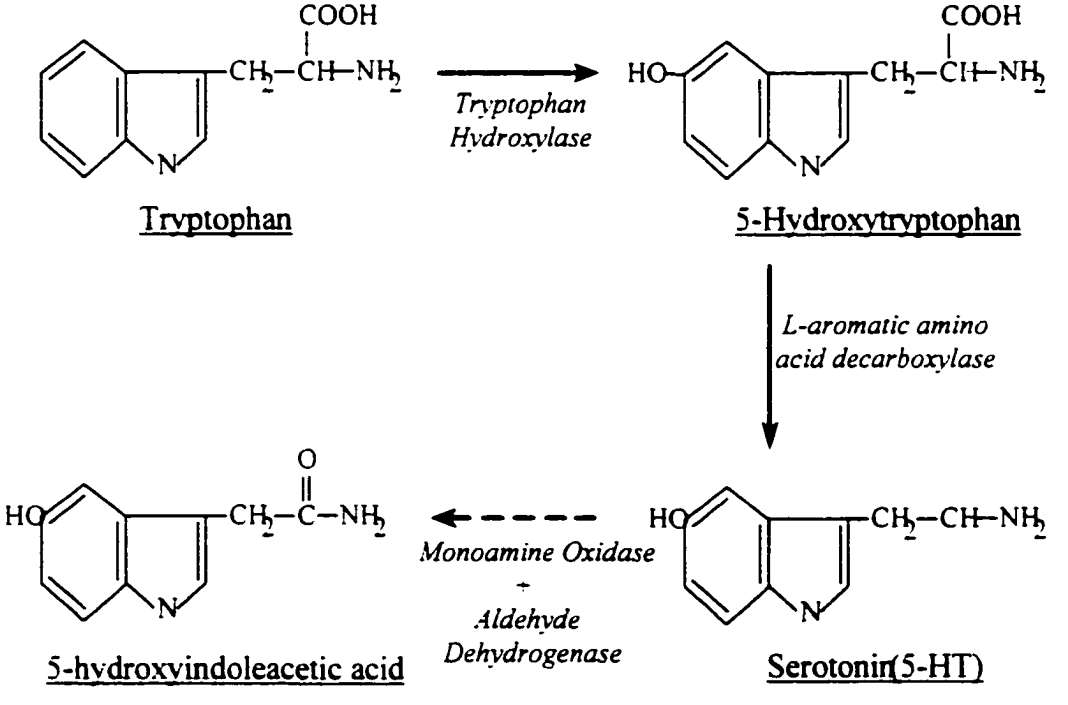

Figure 3. Major Metabolic Pathway for Serotonin Synthesis and Metabolism. *Solid lines* indicate synthetic pathways and *dashed lines* indicate metabolic pathways.

HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} subtypes and is negatively coupled to adenyl cyclase. The 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors are localized both pre- and postsynaptically and act as autoreceptors (Davidson & Stamford, 1995). The 5-HT_{1A} subtype is expressed in the hippocampus, cortex, septum, amygdala, and raphe nuclei, while the 5-HT_{1B}, and 5-HT_{1D} subtypes are found primarily in extrapyramidal regions, including the substantia nigra, globus pallidus, and striatum (Vergé et al., 1986). The 5-HT_2 family of receptors comprises the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} subtypes, which stimulate phospholipase C (Julius et al., 1990). The 5-HT_{2A} receptor is abundant in the claustrum and frontal cortex

Table 1. Current nomenclature and characteristics of 5-HT receptor subtypes

Family	Subtypes	Effector System	Cellular Action
5-HT_1	5-HT_{1A} 5-HT_{1B} 5-HT_{1D} 5-HT_{1E} 5-HT_{1F}	G_i protein	↓cAMP
5-HT_2	5-HT_{2A} 5-HT_{2B} 5-HT_{2C}	phospholipase C	IP_3/DG
5-HT_3		Open Cl^- channel	Depolarization
5-HT_4		G_S protein	↑cAMP
5-HT_5	5-HT_{5A} 5-HT_{5B}	G_S protein	↑cAMP
5-HT_6		G_S protein	↑cAMP
5-HT_7		G_S protein	↑cAMP

Note: cAMP = adenosine 3',5'-cyclic monophosphate; DG = diacylglycerol; IP_3 = inositol triphosphate.

(Pazos & Palacios, 1985), while the 5-HT_{2C} receptor is widely expressed in the piriform cortex, ventral striatum, thalamus, amygdala, substanti nigra, and central gray (Mengod et al., 1990). In contrast, expression of the 5-HT_3 subtype, which is the only 5-HT receptor directly coupled to a ligand-gated Cl^- channel, is limited to the medulla (Maricq et al., 1991). The 5-HT_4, 5-HT_6, and 5-HT_7 receptors stimulate adenyl cyclase, while the effector systems of the 5-HT_{5A} and 5-HT_{5B} receptors remain unknown. The 5-HT_4 receptor is expressed in numerous brain regions, including the striatum, cortex, septum, hippocamopus, amygdala, and substantia nigra (Vilaró et al., 1996).

Putative Functions. Several theorists have postulated that the brain 5-HT system plays a prominent role in behavioral inhibition (Gray, 1982; Soubrié, 1986). These theories attempt to account for the increased responding in various animals following depression of central 5-HT transmission, although they propose slightly different roles for 5-HT. Soubrié (1986) suggests that the primary function of the brain 5-HT system is to tonically suppress behavior. Consequently, reducing central 5-HT transmission produces a shift in behavior from inhibition to facilitation of responding, thereby releasing behaviors that are generally potently inhibited (e.g., aggression) (Soubrié, 1986). In contrast, Gray (1982) proposes that central 5-HT sensitizes animals to signals or cues associated with punishment as part of a general behavioral inhibition system. According to this theory, lowering central 5-HT transmission reduces the saliency of punishment cues, thereby leading to the increased responding observed during punishment and non-reward contingencies. Despite the differences between the two theories, both predict that humans with low central 5-HT activity will exhibit excessive impulsive, antisocial, and maladaptive behavior (Gray, 1982; Soubrié, 1986).

DIMINISHED CENTRAL 5-HT FUNCTION AND AGGRESSIVE BEHAVIOR

The inverse relationship between central 5-HT function and aggressive behavior is among the most consistently reported findings in modern biological psychiatry. Diminished central 5-HT function seems to be a risk factor for aggressive and violent behavior and a variety of psychopathological correlates, including poor impulse control, antisocial behavior, and alcohol abuse. Support for this hypothesis comes from both experimental and correlational studies in animals and human adults.

Studies in Animals. Experimental manipulations in animals that deplete central 5-HT stores increase aggressive behavior and alcohol consumption in several behavioral paradigms, while interventions that augment 5-HT neurotransmission reduce aggression (reviewed in Soubrié, 1986). Electrolytic lesions of the 5-HT-rich raphe nuclei (Waldbillig, 1979), chemical lesions of central 5-HT systems with 5,6- or 5,7-dihydroxytryptophan (Vergnes et al., 1988), and inhibition of 5-HT synthesis with para-chlorophenylalanine (Vergnes et al., 1986) or a tryptophan-deficient diet (Kantak et al., 1980) decrease the latency to attack and increase the number and intensity of attacks in rodents in several aggression paradigms. Conversely, aggressive responding is reduced by acute administration of the 5-HT releaser/reuptake-inhibitors fenfluramine (File & Guardiola-Lemaitre, 1988) and fluoxetine (Kostowski et al., 1984) and by a tryptophan-enhanced diet (Kantak et al., 1980). Several selective 5-HT agonists have also been found to reduce aggression in rats and mice. The presynaptic 5-HT_{1A} agonists ipsapirone and 8-hydroxy-2-(di-*n*-prpylamino) (8-OH-DPAT) and the postsynaptic 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI) all reduce aggressive responding,

but also decrease social and exploratory activity (Oliver & Mos, 1992). In contrast, the mixed 5-$HT_{1A/1B}$ agonists eltoprazine and RU24969 dose-dependently reduce aggressive responding without affecting social or exploratory activity (Mos et al., 1992; Oliver & Mos, 1992; Sijbesma et al., 1991). In contrast, deletion of the gene encoding MAO-A generates mice that are more aggressive as adults than their wild-type counterparts, but that also exhibit obvious sensorimotor and behavioral abnormalities (Cases et al., 1995). These mice exhibit elevated concentrations of both 5-HT and norepinephrine in the brain, making interpretation of these findings difficult.

Strong correlative evidence of a link between low central 5-HT and aggression has come from a series of longitudinal studies of free-ranging male nonhuman primates living in a naturalistic setting (Higley et al., 1992a, 1996a; 1996b, Mehlman et al., 1992, 1994, 1997). These studies consistently reported an inverse correlation between cerebrospinal fluid (CSF) concentrations of 5-HIAA, and aggressive and antisocial behaviors. Prepubescent male primates with low CSF 5-HIAA exhibit excessive levels of severe and unrestrained aggression that readily escalates and places them at risk for injury (Higley et al., 1992a, 1996a; Mehlman et al., 1994). These primates also display other impulsive and risk-taking behavior, such as entering food-baited traps and jumping long distances at dangerous heights in the treetops (Higley et al., 1996a). Socially, primates with low CSF 5-HIAA are less competent, display less affiliative behavior, rank low in social dominance, tend to be isolated and ostracized, and consequently are expelled from the social group at an early age (Mehlman et al., 1995). This maladaptive behavioral repertoire likely accounts for the high rate of premature mortality due to violence among primates with low CSF 5-HIAA (Higley et al., 1996b). In contrast, high

CSF concentrations of 5-HIAA in nonhuman primates are associated with restrained aggression and a range of competitive behaviors necessary for the achievement of social dominance (Mehlman et al., 1995). The inverse relationship between maladaptive aggression and CSF 5-HIAA in primates is masked during the mating season by the normative increase in both CSF 5-HIAA levels and severe aggression during male-male competition over access to reproductive females (Mehlman et al., 1997).

Parallel longitudinal studies of nonhuman primates raised in laboratory settings confirmed the inverse relationship between CSF 5-HIAA and aggressive behavior, and demonstrated that early social experience impacts on this relationship (Clarke et al., 1996; Higley et al., 1991). Primates who were reared for the first six months of life with their peers only (i.e., maternal deprivation) had lower CSF 5-HIAA concentrations and displayed more severe and unrestrained aggression and less competent social behavior in infancy and young adulthood than siblings reared in mother-infants dyads for the first six months of life (Clarke et al., 1996; Higley et al., 1991). Moreover, among peer-reared primates, those with low CSF 5-HIAA levels exhibited greater alcohol consumption, higher intrinsic tolerance to alcohol, and greater rates of aggression during intoxication (Higley et al., 1996c; Higley & Bennett, 1999).

The mechanisms that determine interindividual differences in CSF 5-HIAA concentrations in nonhuman primates have been largely delineated. The interindividual differences in CSF 5-HIAA levels are believed to reflect variability in presynaptic neuronal function (Heinz et al., 1998). CSF 5-HIAA concentrations decline with age in young male primates, starting in infancy and eventually leveling off as primates enter young adulthood (Clarke et al., 1996; Higley et al., 1991, 1992b). Yet, interindividual

differences in CSF 5-HIAA levels remain relatively stable over time and across settings in these primates, suggesting that central 5-HT function is associated with a behavioral trait. Consistent with this hypothesis, the CSF 5-HIAA response to the stress of social separation was also relatively stable over extended periods of time (Higley et al., 1991, 1992b). Moreover, CSF 5-HIAA concentrations are heritable and partially determined by pedigree (Clarke et al., 1995; Higley et al., 1993), with paternal and maternal genetic contributions accounting for over half of the variance in CSF 5-HIAA levels among young male primates (Higley et al., 1993). However, as demonstrated by the peer-rearing paradigm, CSF 5-HIAA concentrations are not immutable from birth, but rather, are modifiable by early experiential factors.

Together, these data indicate that an inverse relationship between central 5-HT activity and aggression exists in several animal species. The expression of aggressive behavior in rodents and primates can be increased or decreased by manipulations of central 5-HT function. Moreover, reduced central 5-HT in primates is associated not only with aggression, but also with a variety of interrelated maladaptive behaviors, including poor impulse control and alcohol abuse. Diminished central 5-HT activity is stable over time and seems to reflect a behavioral trait.

Studies in Human Adults. Neurochemical studies in human adults have also consistently reported either inverse correlations between indices of 5-HT and measures of aggression or reduced 5-HT function in a wide array of aggressive individuals. These studies have examined both peripheral (e.g., platelet) and central (e.g., CSF) measures of 5-HT function, and have begun to combine some of these measures with brain imaging techniques to provide greater anatomical specificity. Finally, molecular studies have

identified several candidate genes in adult aggression.

Peripheral indices of 5-HT (e.g., platelet) have been studied as models of central 5-HT function due to similarities in uptake, storage, and release processes (Stahl, 1977). However, these studies have largely yielded inconsistent results regarding 5-HT function in aggressive adults. Among the six studies that examined platelet [^{3}H]imipramine and [^{3}H]paroxetine binding density (B_{max}), both putative indices of 5-HT transporter sites: two reported reduced binding in aggressive inpatients with mental retardation (Marazziti & Conti, 1991; Marazziti et al., 1996); one found elevated binding in violent offenders and hostile schizophrenics (Sarne et al., 1995); one reported inverse correlations with aggression in patients with personality disorders (Coccaro et al., 1996a); and two observed no relationship with ratings of aggression in healthy volunteers (Castrogiovanni et al., 1994; Coccaro et al., 1996a). In addition, platelet 5-HT uptake (V_{max}) was decreased in outpatients with episodic aggression (Brown et al., 1989) and platelet MAO activity was reduced in psychopathic criminals (Lidberg et al., 1985), together suggesting elevated levels of synaptic 5-HT in aggressive individuals. Consistent with such a hypothesis, whole blood 5-HT was elevated in violent relative to nonviolent men in an epidemiological sample (Moffitt et al., 2000). Finally, platelet 5-HT_{2A} receptor binding was correlated with self-reports of aggression in patients with personality disorders, but not healthy volunteers (Coccaro et al., 1997b). These findings point to possible abnormalities in platelet processes thought to reflect presynaptic neuronal functions, although the data are far from conclusive. Further, the relevance of peripheral indices to 5-HT function in the brain is not altogether clear.

Studies assessing CSF 5-HIAA in aggressive adults are plentiful and generally

support the hypothesized inverse relationship between central 5-HT function and aggression. Reduced CSF 5-HIAA concentrations were reported in: impulsive murderers (Lidberg et al., 1985); alcoholic offenders with APD and/or intermittent explosive disorder (Virkunnen et al., 1994); aggressive inpatients with mixed Axis I disorders (Stanley et al., 2000); violent volunteers (Hibbeln et al., 1998); aggressive alcoholics (Limson et al., 1991); violent criminal offenders (Linnoila et al., 1983; Virkunnen et al., 1989, 1996); and arsonists (Virkunnen et al., 1987, 1989, 1996). Furthermore, low CSF 5-HIAA concentration was associated with recidivism in the latter two samples (Virkunnen et al., 1989, 1996). However, not all studies of CSF 5-HIAA in aggressive adults are so consistent. Among studies of CSF 5-HIAA in patients with personality disorders, two initially reported negative correlations with lifetime history of aggression (Brown et al., 1979, 1982), but five more recent studies found no relationship with several measures of aggression (Coccaro 1992; Coccaro et al., 1997c, 1997d, 1998; Gardner et al., 1990). Moreover, CSF 5-HIAA concentration was inversely correlated with hostility in one study of healthy volunteers (Roy et al., 1988), but positively related to extroverted aggression in another (Moller et al, 1996).

Neuroendocrine challenge studies have provided some of the strongest evidence of a link between reduced central 5-HT function and aggressive and antisocial behavior in adults. The rise in plasma hormone levels following acute administration of various 5-HT agonists afford dynamic measures of central 5-HT activity in the hypothalamic-pituitary axis (Fuller, 1992). The majority of these studies assessed the prolactin response to the 5-HT releasing and reuptake-inhibiting agent fenfluramine, which reflects combined pre- and postsynaptic 5-HT activity. The prolactin response to fenfluramine

was inversely correlated (range of r: -0.40 to -0.57) with aggression in personality disordered patients (Coccaro et al., 1989, 1995, 1996b, 1997c, 1997d; Stein et al., 1996), veterans with post-traumatic stress disorder (Davis et al., 1999), and healthy volunteers (Manuck et al., 1999). Moreover, compared to controls, the prolactin response to fenfluramine was blunted in convicted murderers with APD (O'Keane et al., 1992), criminal offenders with personality disorders and/or alcohol abuse (Dolan et al., 2001), depressed patients with anger attacks (Fava et al., 2000), and impulsive volunteers (Evans et al., 2000). However, the prolactin response to fenfluramine was positively related to aggression in polysubstance abusers (Fishbein et al., 1989), which may reflect the effects of long-term substance abuse. The partial 5-HT agonist meta-chlorophenylpiperazine (m-CPP) has been used to assess the functional integrity of postsynaptic 5-HT receptors in aggressive adults. Prolactin responses to m-CPP were inversely related (range of r: -0.32 to -0.40) to measures of aggression and hostility in substance abusers with APD (Moss et al., 1990), abstinent alcoholics (Buydens-Branchey et al., 1997; Handelsman e al., 1996), and patients with personality disorders (Coccaro et al., 1997d), but not in patients with major depression and/or panic disorder (Wetzler et al., 1991). Finally, blunted cortisol responses to the 5-HT_{1A} agonist ipsapirone in aggressive personality disordered patients (Coccaro et al., 1995) and aggressive volunteers (Cleare & Bond, 2000) compared to controls suggest a role for postsynaptic 5-HT_{1A} receptors in aggression.

Several recent studies have used brain imaging techniques in combination with pharmacological agents to localize abnormalities in central 5-HT activity in aggressive adults. Positron emission tomography (PET) with [^{18}F]fluorodeoxyglucose (FDG) was

used to study regional glucose metabolism following fenfluramine activation in aggressive personality disordered patients (Siever et al., 1999; Soloff et al., 2000). Reduced glucose responses to fenfluramine were found in medial and orbital prefrontal cortex, anterior cingulate, left parietal lobe, left superior and middle temporal gyri, and caudate nucleus. Brain imaging studies have also yielded evidence of presynaptic 5-HT dysfunction in populations of aggressive and impulsive adults. Patients with borderline personality disorder have reduced 5-HT synthesis in the medial frontal gyrus, anterior cingulate, superior temporal gyrus, and striatum, as measured by PET and the 5-HT precursor analog α-[^{11}C]methyl-L-tryptophan ([^{11}C]MTrp)(Leyton et al., 2001). In addition, single-photon emission computerized tomography (SPECT) with [^{123}I]2β-carbomethoxy-3β-(4-iodophenyl)-tropane ([^{123}I]β-CIT) revealed low 5-HT transporter density in the midbrains of violent offenders (Tiihonen et al., 1997) and abstinent alcoholics (Heinz et al., 1998). Together, these findings suggest that impulsive aggressive adults may have abnormal presynaptic 5-HT activity in the midbrain (i.e. raphe nuclei) that results in altered 5-HT activation of brain regions implicated in aggression (e.g., orbitofrontal cortex).

Newer research approaches have examined the effects of acute and chronic manipulation of 5-HT on aggressive behavior in adults. Acute dietary depletion of tryptophan reduces the availability of tryptophan for conversion to 5-HT and inhibits the synthesis of 5-HT in the brain (Williams et al., 1999). Tryptophan depletion increases aggressive responding on laboratory tests in healthy volunteers (Bjork et al., 1999; LeMarquand et al., 1999; Moeller et al., 1996; Pihl et al., 1995), although this seems to

be primarily accounted for by individuals who are prone to aggression (Bjork et al., 2000; Cleare & Bond, 1995; Dougherty et al., 1999). Conversely, dietary tryptophan enhancement decreases aggressive responding in volunteers (Cleare & Bond, 1995; Moeller et al., 1996). Dose-dependent reductions in aggressive and impulsive responding were also reported following acute fenfluramine in males with criminal and antisocial histories (Cherek & Lane, 1999, 2000). Reductions in aggressive behavior and feelings of anger and irritability have also been reported following continued treatment with selective 5-HT reuptake inhibitors (SSRIs). Treatment with sertraline and fluoxetine reduced aggression in adults with mental retardation (Hellings et al., 1996; Markowitz et al., 1992; McDougle et al., 1998), decreased anger attacks in patients with major depressive disorder (Fava et al., 1993), and reduced aggressive and impulsive behavior and feelings of irritability in patients with various personality disorders (Coccaro et al., 1990, 1997e; Coccaro & Kavoussi, 1997; Cornelius et al., 1991; Kavoussi et al., 1994). Counterintuitively, the anti-aggressive response in personality disordered patients was positively correlated with the prolactin response to fenfluramine, such that individuals with higher prolactin responses had the greatest treatment response (Coccaro et al., 1997e). This finding raises the possibility that those patients with less severe disease, as manifested by a more robust prolactin response, are more drug responsive. Nonetheless, these data suggest that the relationship between diminished central 5-HT and aggression in adults is direct and causal.

Several parallel lines of research (e.g., family, adoption, and twin studies) indicate that aggressive behavior is heritable and partially determined by genetic factors (Cadoret et al., 1995; Coccaro et al., 1997a; Lahey et al., 1988). Various 5-HT system

genes have been studied as potential candidate genes in aggression, but so far the results are inconclusive. The evidence is strongest linking aggressive behavior with a biallelic polymorphism in intron 7 of the gene encoding tryptophan hydroxylase (TPH) (Mannuck et al., 1999; New et al., 1998), the rate-limiting enzyme in 5-HT synthesis. Yet, aggression was alternatively associated with the L allele of this polymorphism in a small sample of personality disordered males (New et al., 1998) and with the U allele in a large community sample (Mannuck et al., 1999). Variable number tandem repeat (VNTR) regulatory polymorphisms in the MAOA gene were also associated with individual differences in aggression in a community sample of males (Mannuck et al., 2000). High trait aggression and impulsivity were linked with the 2- and 3-repeat alleles of the MAOA gene, which mediate enhanced transcriptional activity in MAOA promoter constructs (Sabol et al., 1998). In addition, a single point mutation in exon 8 of the MAOA gene that produces a deficiency in enzymatic activity was associated with a behavioral phenotype characterized by impulsive aggression, arson, attempted rape, and exhibitionism in affected males of a large kindred (Brunner et al., 1993). However, the polymorphisms in TPH and MAOA were not associated with aggression in schizophrenia or schizoaffective disorder (Nolan et al., 2000). While 5-HT genes seem to be involved in the manifestation of aggression in human adults, the data are inconsistent with regard to the specific genotype. Further research and replication is clearly necessary.

In sum, a large body of clinical research in adult males suggests a causal link between diminished central 5-HT function and a behavioral phenotype characterized by aggressive, impulsive, and antisocial behavior and alcohol abuse. These data point to reductions in both presynaptic (e.g., 5-HT synthesis) and postsynaptic (e.g., 5-HT_{2A}

receptors) components of 5-HT activity that have been preliminarily localized to the midbrain raphe nuclei and their neocortical target regions. However, no genotype has yet been identified to account for this behavioral phenotype. More generally, the data indicate that reduced central 5-HT activity is not specific to any one psychiatric disorder, but rather seems to be related to an aggressive trait that exists across diagnostic categories.

Studies in Children. Diminished central 5-HT function has also been postulated to play a role in the manifestation of aggressive behavior in boys. However, direct assessments of central nervous system function are invasive and more difficult to conduct in children. Therefore, developmental studies of the relationship between 5-HT and aggression have relied primarily on peripheral measures. Ethical considerations precluded the inclusion of adequate control groups in many of these studies, which further constrains the interpretation of the results.

Studies that employed peripheral indices to assess the relationship between 5-HT and aggression in children are plentiful, but highly inconsistent in their findings. Whole blood 5-HT concentrations were elevated in impulsive adolescent inpatients compared to matched controls (Askenazy et al., 2000) and were positively correlated with measures of violence and conduct disorder (CD) in incarcerated adolescents (Pliszka et al., 1988; Unis et al., 1997). Yet, other studies have reported that whole blood 5-HT levels were inversely correlated with ratings of aggression in children with obsessive-compulsive disorder and in pubescent children of alcoholics (Twitchell et al., 2000; Hanna et al., 1995) and were unrelated to measures of aggression and CD in children with ADHD (Cook et al., 1995; Spivak et al., 1999). Studies that examined platelet measures of 5-HT

have yielded similarly inconsistent findings. Low platelet MAO activity in juvenile delinquents predicted criminal outcome in adulthood (Alm et al., 1996), but was unrelated to aggression in boys with disruptive behavior disorders (DBD), including ADHD and CD (Stoff et al., 1989). In addition, platelet [^{3}H]imipramine binding density (B_{max}) was negatively correlated with aggression in impulsive aggressive children (Birmaher et al., 1990) and aggressive adolescent inpatients (Stoff et al., 1987), and was lower in boys with comorbid ADHD and CD compared to matched controls (Stoff et al., 1987), although the latter finding was not replicated in a larger sample (Stoff et al., 1991). Further, platelet [^{3}H]paroxetine binding was unrelated to measures of violence in incarcerated adolescents (Unis et al., 1997). Finally, platelet 5-HT_{2A} receptor binding density (B_{max}) was inversely related to parental risk factors in children at risk for antisocial behavior (Pine et al., 1996), and was reduced in incarcerated adolescents compared to controls (Blumensohn et al., 1995), but was unrelated to aggression in children with ADHD (Pornnoppadol et al., 1999). The inconsistent findings using peripheral measures of 5-HT function makes extrapolation of the data to neuronal function virtually impossible.

The invasive nature of obtaining CSF has precluded extensive research of central 5-HT metabolites in aggressive children, and the few studies conducted have yielded conflicting results. An early study found that a heterogenous sample of highly aggressive boys with DBD had lower CSF 5-HIAA concentrations than boys with obsessive-compulsive disorder (Kruesi et al. 1990). Moreover, CSF 5-HIAA was inversely correlated with ratings of aggression (Kruesi et al. 1990) and predicted aggression at two-year follow-up in the boys with DBD (Kruesi et al. 1992). However, the same laboratory

later reported a positive association between CSF 5-HIAA levels and ratings of aggression in boys selected for the presence of ADHD (Castellanos et al. 1994). Systematic comparison of the samples in the two studies indicated that the second sample was younger, less aggressive, and more hyperactive. Any of these variables may have accounted for the difference in the direction of the findings.

A more recent series of studies that examined 5-HIAA concentrations in CSF drawn from newborns for medical indications reported low concentrations in newborns with a family history of APD (Constantino et al. 1997). Moreover, newborns with low CSF 5-HIAA levels were less sociable at nine months of age (Constantino & Murphy, 1996), and exhibited greater externalizing behavior at 30 months of age (Clarke et al., 1999). However, these associations were weak and disappeared altogether when the prevalence of APD in relatives was controlled for.

Studies using the less invasive fenfluramine challenge procedure to examine central 5-HT function in children have also yielded discrepant results. The prolactin response to fenfluramine was positively correlated with ratings of aggression in a non-referred sample of siblings of adjudicated adolescents (Pine et al., 1997), and was augmented in aggressive relative to nonaggressive boys with ADHD (Halperin et al., 1994). However, the latter finding was not replicated in a second sample of boys with ADHD (Halperin et al., 1997a). Systematic comparison of the two ADHD samples indicated that they differed only in age and raised the possibility that the relationship between central 5-HT and aggression in boys with ADHD may vary as a function of age. In contrast, the prolactin response was unrelated to ratings of aggression and CD symptom counts in small samples of prepubertal and adolescent males with DBD (Stoff

et al., 1992).

The variable results of studies assessing central indices of 5-HT function in children suggest that the association between aggression and central 5-HT function in children is not simply characterized by the inverse relationship typically found in adults. Data in children clearly indicate that aggressive children are neither uniformly "high" nor uniformly "low" with regard to central 5-HT function. As such, the important question in children does not seem to be "Do aggressive children have low (or high) 5-HT function relative to non-aggressive children?" Rather, the key question becomes "What is the significance of low (or high) central 5-HT function in aggressive children?"

Several investigators have attempted to answer this question by reconciling the differences across the child studies of central 5-HT function and aggression (Castellanos et al., 1994; Halperin et al., 1997a; Pine et al., 1997). These analyses have suggested that the inconsistency of central 5-HT findings in children may be attributable to: 1) diagnostic differences across the samples; 2) developmental differences across the samples; or 3) the heterogeneity of aggressive children. In addition, reports of seasonal variations in measures of 5-HT function in humans suggest that similar seasonal effects may account for some of the variability among the child studies of central 5-HT activity and aggression.

The heterogeneous nature of aggression in children might account for the inconsistency of central 5-HT findings in children. Only a small proportion of aggressive boys progress to serious violence as adolescents and adults (Loeber et al., 1993). Numerous factors influence whether or not a boy progresses towards violence (reviewed in Farrington & Loeber, 2000), and may obscure the role that low central 5-HT activity

plays in this escalation. Yet, little is known regarding the extent to which low central 5-HT function is associated with these variables, in particular familial aggression. Familial aggression and sociopathy may be associated with reduced central 5-HT function (Coccaro et al., 1994; Halperin et al., 1997b).

The differences in age and the proportion of subjects with ADHD across the samples in the child studies of central 5-HT function raises the possibility that the association between central 5-HT and aggression varies as a function of age or ADHD. Specifically, central 5-HT activity: a) was greater in young aggressive boys relative to young nonaggressive boys, but did not differ among older boys (Halperin et al., 1997a); and b) was positively correlated with aggression in samples of boys with ADHD (Castellanos et al., 1994; Pine et al., 1997), but inversely correlated in samples that contained fewer cases of ADHD (Kruesi et al., 1990, 1992).

Seasonal fluctuations in 5-HT activity might also account for some of the variability among the child studies of central 5-HT activity. Seasonal variations in 5-HT activity have been described in adults, such that 5-HT is elevated in Autumn and reduced in Spring (Cappiello et al., 1996; Maes et al., 1995). However, evidence of seasonal variations in 5-HT activity in children is scant, and suggests that 5-HT seasonality in children varies as a function of psychiatric status (Pine et al., 1995). Group-specific seasonal differences in 5-HT could account for the variability and overlap between aggressive and nonaggressive boys in the child studies of central 5-HT.

SUMMARY AND CONCLUSIONS

The high stability coefficients of aggression over time reported in the literature do not reflect the considerable individual discontinuity in aggression during childhood. In fact, numerous young boys present with minor aggression, but only a small proportion progress to fighting, and even a smaller subgroup escalates to serious violence. The mechanisms mediating this adverse developmental trajectory are poorly understood, but seem to involve an interaction of psychosocial and/or environmental variables with biological factors. As such, the identification of biological risk factors during early childhood may be an important step toward early intervention.

Considerable research has established low brain 5-HT activity as a biological risk factor for aggressive behavior in animals and human adults. Diminished central 5-HT function is associated with a behavioral phenotype characterized by aggressive, impulsive, and antisocial behavior. This relationship seems to reflect a behavioral trait that is stable over time and exists across species in animals and across diagnostic categories in human adults. Central 5-HT function has also been postulated to play a role in the manifestation of childhood aggression, but the few studies conducted in children have yielded inconsistent results. Data across laboratories indicate that aggressive children are not uniformly "high" or "low" with regard to central 5-HT function. Yet the significance of this variability remains unclear. Careful review of the research indicates that the inconsistency of central 5-HT findings in children may be attributable to diagnostic or developmental differences across the samples or to familial factors associated with aggression in children. Other data suggest that the variability among the studies may be accounted for by factors outside the sample, such as seasonal variations in 5-HT function. This series of three studies examined the impact of 1) seasonal

fluctuations in 5-HT indices, 2) age and the presence of ADHD, and 3) familial aggression and sociopathy on the relationship between central 5-HT and aggression in prepubertal boys.

RATIONALE FOR STUDIES

STUDY ONE: LACK OF SEASONAL RHYTHMS IN CENTRAL 5-HT ACTIVITY IN BOYS

Seasonal fluctuations in 5-HT activity might account for some of the variability among the child studies of central 5-HT activity. Circannual seasonal variations have been described in indices of 5-HT activity in various populations of adults, such that 5-HT activity is likely to be elevated in the late Summer and Fall and reduced in the Spring (Brewerton et al., 1988; Cappiello et al., 1996; Maes et al., 1995). This seasonality in 5-HT has been linked to such weather variables as ambient temperature, relative humidity, and air pressure (D'Hondt et al., 1996). However, evidence of seasonal variations in 5-HT activity in children is scant and inconsistent. For example, one research group reported a peak in platelet [^{3}H]imipramine binding during the summer in adolescent suicide attempters (Pine et al., 1995), but not in adolescents with CD (Pine et al., 1995). These findings raise the possibility that 5-HT seasonality in children varies as a function of psychiatric status, which could account for some of the variability and overlap between aggressive and nonaggressive boys in the current sample (Halperin et al., 1994, 1997a).

The aim of Study 1 was to determine whether distinct seasonal fluctuations in central 5-HT activity exist among aggressive and nonaggressive boys with ADHD. It was predicted that aggressive boys would exhibit either a larger summertime and autumnal peak or a smaller springtime trough in central 5-HT activity than nonaggressive

boys, and that these seasonal effects would mask the inverse relationship between central 5-HT activity and aggression in the sample.

STUDY TWO: THE IMPACT OF AGE AND ADHD ON THE RELATIONSHIP BETWEEN CENTRAL 5-HT ACTIVITY AND AGGRESSION IN BOYS

Attempts to reconcile the differences across the child studies of central 5-HT function and aggression indicated that the samples differed in age (Castellanos et al., 1994; Halperin et al., 1997a). Among the six studies, central 5-HT activity was positively correlated with aggression in the three youngest samples (Castellanos et al., 1994; Halperin et al., 1994; Pine et al., 1997), not associated with aggression in the two slightly older prepubertal and adolescent samples (Halperin et al., 1997a; Stoff et al., 1992), and inversely related to aggression in the oldest sample (Kruesi et al., 1990, 1992). These findings raise the possibility that age-related changes in central 5-HT activity may explain the discrepancy across the studies of 5-HT and childhood aggression, as well as provide a link between the child and adult literature.

Another possible explanation for the inconsistent findings posits differences in comorbid disorders across samples, specifically in the proportion of subjects with ADHD (Castellanose et al., 1994). Among the three studies that reported a positive correlation between central 5-HT function and aggression, two were selected specifically for ADHD (Castellanos et al., 1994; Halperin et al., 1994), and the third consisted of a non-referred sample with a large number of boys diagnosed with ADHD (Pine et al., 1997). In contrast, the two studies that reported an inverse or no association between 5-HT and

aggression were conducted in more heterogenous samples of boys with DBD that contained fewer cases of ADHD (Kruesi et al., 1990, 1992; Stoff et al., 1992). Thus, the presence of ADHD may influence the relationship between central 5-HT function and aggression in children.

This study examined whether age or the presence of ADHD influenced the relationship between central 5-HT function and aggression in nonaggressive boys with ADHD, aggressive boys with ADHD, and aggressive boys without ADHD. It was hypothesized that central 5-HT responsivity would be: a) lowest in aggressive boys without ADHD and highest in aggressive boys with ADHD, with nonaggressive boys with ADHD falling in between; and b) greater in young aggressive boys relative to young nonaggressive boys, but not differ among older boys.

STUDY THREE: FAMILIAL CORRELATES OF CENTRAL 5-HT ACTIVITY IN BOYS

A more compelling hypothesis to account for the variability across the child studies of central 5-HT function posits the existence of distinct subgroups of aggressive boys with regard to central 5-HT activity. Only a small proportion of aggressive boys progress to serious violence as adolescents and adults (Loeber et al., 1993). Social, familial, peer, and individual factors all influence whether or not a boy progresses towards violence (reviewed in Farrington & Loeber, 2000), and may obscure the role that central 5-HT dysfunction plays in this escalation. Yet, little is known regarding the extent to which these variables are associated with central 5-HT function in boys. Many

aggressive boys will desist in their aggression later in life, and may not have the same neurobiology as those boys whose aggression is likely to escalate.

Familial sociopathy and aggression are important risk factors for the progression of childhood aggression (Cadoret et al., 1995; Frick et al., 1992). It may also be that familial aggression and sociopathy are associated with reduced central 5-HT function. Studies in child and adult samples have reported low CSF 5-HIAA concentrations in individuals with high rates of familial criminality and APD (Constantino et al., 1997; Virkunnen et al., 1996). Further, a blunted prolactin response to fenfluramine was associated with high rates of aggression in the relatives of adults with personality disorders (Coccaro et al., 1994) and with parental history of aggression in aggressive children (Halperin et al., 1997b). These data raise the possibility that aggressive boys with low central 5-HT function represent an endophenotype (i.e., a refined phenotype that is a candidate for a highly heritable phenomenon).

The current study tested this hypothesis by examining the incidence of aggressive and antisocial behaviors in first and second degree relatives of aggressive and nonaggressive children who were further subdivided based upon central 5-HT responsivity. It was postulated that the aggressive children with reduced 5-HT function would have a higher frequency of relatives with aggressive and antisocial behaviors compared to both nonaggressive children and aggressive children with higher 5-HT activity.

GENERAL RESEARCH DESIGN AND METHODS

SUBJECTS.

The participants were seven to eleven year-old boys who were recruited as part of two separate NIMH-funded studies of central 5-HT function and childhood aggression conducted from 1990 to 1994 and from 1994 to 1997. As shown in Table 2, the two independent samples of boys (heretofore referred to as Sample 1 and Sample 2) did not differ in age. The boys were all referred to the Child Behavior Disorders Research Team of the Mount Sinai School of Medicine for clinically significant levels of disruptive behavior (i.e., inattention, hyperactivity-impulsivity, aggression, delinquency, and/or defiance). Referrals came from a variety of sources throughout the New York-New Jersey Metropolitan Area. A large number of boys were referred from the Child Psychiatry Outpatient Clinic of the Mount Sinai Medical Center, which serves an urban and largely minority population. Many participants were also referred by public, parochial, and private schools in urban and suburban areas of New York and New Jersey. Referrals from primary care physicians and support and advocacy groups (e.g., Children and Adults with Attention Deficit Disorder [CHADD]) also provided several participants. Finally, the parents of several participants contacted the Child Behavior Disorders Research Team directly after hearing presentations on ADHD by team members. Thus, while the sample was predominately urban and of lower socioeconomic status, boys from suburban areas and from all socioeconomic levels were represented. With regard to ethnicity, the sample is quite diverse and largely reflects the diversity of the New York

metropolitan area. Among the sample, 23% are Caucasian, 35% are African-American, 38% are of Hispanic decent, and 4% are of Asian or mixed ancestry. There was no difference in ethnicity between the two samples ($p > 0.10$). However, the two samples did differ in Full Scale IQ score. This difference will be addressed in more detail in the following section on cognitive measures.

All participants were free of medication for at least 30 days prior to the biological

Table 2. Demographic, psychometric, and biological characteristics of Samples 1 and 2

	Sample 1 ($n = 54$)		Sample 2 ($n = 46$)		Analysis	
Variable	Mean	S.D.	Mean	S.D.	t	*p*
Age (in years)	9.1	1.3	9.4	1.3	1.19	n.s.*
Socioeconomic Status	32.0	14.9	30.0	14.3	0.66	n.s.
Full Scale IQ	97.9	12.6	92.7	12.5	2.03	0.04
Child Behavior Checklist						
Social Withdrawal	59.6	10.6	63.4	10.9	1.65	n.s.
Somatic Complaints	58.8	7.4	62.8	11.5	1.97	0.05
Thought Problems	61.1	10.1	64.9	11.8	1.67	n.s.
Anxiety/Depression	64.1	10.7	68.0	12.7	1.57	n.s.
Social Problems	66.8	10.5	68.4	10.9	0.74	n.s.
Attention Problems	71.4	8.4	73.2	10.7	0.87	n.s.
Delinquency	66.6	9.6	69.5	9.1	1.47	n.s.
Aggression	67.4	12.7	74.4	13.7	0.60	n.s.
IOWA Conners						
Inattention/Overactivity	11.1	2.8	11.6	3.1	0.83	n.s.
Aggression/Defiance	8.2	4.6	9.0	4.4	1.09	n.s.
Peak delta prolactin (ng/ml)	13.8	7.6	12.9	7.6	0.66	n.s.

* n.s. = not significant, $p > 0.10$.

procedure. Most of the boys were not receiving any medication at the time of initial contact. Three boys were receiving treatment with psychostimulants for symptoms of ADHD at the time of referral to the study. These three boys were removed from medication by their treating physicians prior to entry into the study either because they were not responding well to the medication and it was determined by their physicians that they would benefit from a re-evaluation off medication, or because the medication was not given over the summer.

General Inclusion/Exclusion Criteria. To be included in the study, participants had to be male, between the ages of seven and 11 years-old, and of prepubertal endocrine status. Girls were studied separately in a parallel investigation with similar procedures (Koda et al., 1996). To recruit a relatively homogeneous sample of boys with DBD, boys with a diagnosis of schizophrenia ($N = 2$), autism, pervasive developmental disorder, or a chronic tic disorder ($N = 6$) were excluded from the study. Similarly, general cognitive ability was assessed to rule out the presence of mental retardation, and no child was accepted if he had a Full Scale IQ below 70 ($N = 17$). To ensure the validity of the biological data, boys with a diagnosed neurological disorder or with chronic medical conditions requiring systemic medication were not enrolled in the study. Finally, children not fluent in English or who were not attending school were not recruited into the study.

Specific Aggression Criteria. The boys were divided into aggressive and nonaggressive groups based on the presence or absence of a *persistent pattern of physically aggressive behavior*. Physical aggression was operationally defined as a persistent pattern during the past six months of at least one of the following items from

the CD symptom list: physically cruel to animals, used a weapon in more than one fight, frequently initiates physical fights, stealing with confrontation of the victim, or physically cruel to people. Non-aggressive symptoms of CD (e.g., lying, truancy) were not used to classify the boys.

All interview and rating scale data were independently reviewed by three board-certified or licensed clinicians who were uninformed about the biological data. The reviewers classified each child as either "aggressive", "nonaggressive", or "equivocal." There was excellent agreement across the three reviewers, with κ coefficients between pairs of raters ranging from 70.1 to 81.8. The few cases for which there were discrepancies across raters were discussed and resolved. This best-estimate procedure was used instead of traditional rating scale cutoffs, because parent and teacher aggression scales tend to assess oppositional and defiant behavior rather than physical aggression, and because there is frequent disparity between parent, teacher, and child reports of aggression. As would be expected, this procedure generated aggressive and nonaggressive groups that differed significantly on both parent and teacher ratings of aggression, as shown in Table 3. Thus, the procedure was not only reliable across evaluators, but generated groups that were consistent with ratings of both parents and teachers. Yet, the procedure for defining the subgroups did yield considerable overlap between aggressive and nonaggressive boys on ratings of aggression (Table 4)

Table 3. Comparison of aggressive and nonaggressive boys from Samples One and Two on ratings of aggression

	Sample 1				Sample 2			
	Nonaggressive (n = 27)		Aggressive (n = 27)		Nonaggressive (n = 13)		Aggressive (n = 33)	
Variable	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Child Behavior Checklist								
Aggressiona	62.0	10.4	73.0	12.6	64.7	12.2	78.2	12.4
Children's Aggression Scale - Parent								
Verbal aggression[a]	5.2	3.8	11.8	6.6	6.0	4.4	10.7	6.2
Aggression against animals & objects	1.7	1.5	2.9	3.3	2.7	2.6	3.1	2.6
Provoked physical aggression[a]	2.1	1.9	4.2	2.6	2.2	2.0	4.4	2.7
Initiated physical aggression[a]	1.3	1.6	3.8	2.7	1.5	2.0	3.5	2.5
IOWA Conners								
Aggression/Defiance[a]	5.3	3.6	11.2	3.4	6.9	5.7	9.8	3.5
Children's Aggression Scale - Teacher								
Verbal aggression[a]	2.0	1.7	7.1	3.2	2.1	1.8	5.5	2.9
Aggression against animals & objects[a]	0.4	0.7	2.4	1.4	0.6	0.9	1.8	1.2
Provoked physical aggression[a,b]	0.7	0.7	2.6	1.7	0.4	0.4	1.8	1.3
Initiated physical aggression[a]	0.5	0.7	2.4	1.2	0.3	0.4	2.0	1.1

[a] Significant main effect for aggression-group, $p < 0.001$.
[b] Significant main effect for sample, $p < 0.05$.

Table 4. Overlap between aggressive and nonaggressive boys on ratings of aggression

	Nonaggressive (n = 40)		Aggressive (n = 60)	
Variable	Min.	Max.	Min.	Max
Child Behavior Checklist				
Aggression	50.0	90.0	55.0	100.0
Children's Aggression Scale - Parent				
Verbal aggression	0.0	16.7	0.0	25.1
Aggression against animals & objects	0.0	7.3	0.0	11.0
Provoked physical aggression	0.0	7.4	0.0	10.4
Initiated physical aggression	0.0	7.4	0.0	10.0
IOWA Conners				
Aggression/Defiance	0.0	15.0	0.0	15.0
Children's Aggression Scale - Teacher				
Verbal aggression	0.0	5.8	0.0	11.0
Aggression against animals & objects	0.0	3.1	0.0	5.9
Provoked physical aggression	0.0	2.3	0.0	5.6
Initiated physical aggression	0.0	2.4	0.0	5.3

MEASURES

The two NIMH-funded investigations of central 5-HT function and childhood aggression used similar assessment procedures. However, some of the assessment measures varied slightly between the investigations. Some tests, scales, and instruments employed in the second investigation were newer or revised versions of those used in the first investigation. The differences between the two investigations will be addressed in the following section.

Diagnostic Measures. Child psychiatric assessments were based on information obtained from parents, teachers, and children. The parents of each child were interviewed using the Diagnostic Interview Schedule for Children (DISC). The DISC is a highly structured and comprehensive diagnostic interview that can be reliably administered by a trained non-clinical interviewer. Diagnoses of DBD and other psychiatric disorders were made based on parental responses to the DISC. Parental responses to the DISC were also used to determine the presence of any exclusionary disorders. Parents of the boys in Sample 1 were interviewed with version 2.1P of the DISC (Shaffer et al., 1989), which reflects DSM-III-R diagnostic criteria, while the parents of boys in Sample 2 were administered version 2.3 of the DISC (Shaffer et al., 1996), which incorporates DSM-IV criteria. The rates of ADHD, CD, oppositional defiant disorder (ODD), and mood disorders did not differ between the two samples and are presented for comparison in Table 5. However, the boys in Sample 2 had a significantly greater rate of social phobia, but not of anxiety disorders in general. The children were also administered a mental status examination designed to uncover the presence of any exclusionary diagnoses.

Parents and teachers also rated the severity of various disruptive behaviors exhibited by the boys. The parents of participants completed the Child Behavior Checklist (CBCL) (Achenbach, 1991). The CBCL consists of a 113-item checklist that is highly reliable and generates standardized scores for a broad range of behavioral dimensions including hyperactivity, aggression, and delinquency. The teachers of participants rated the boys using the IOWA Conners Teacher Questionnaire (IOWA) (Loney & Milich, 1982), which is made up of 10 items from the Conners Teacher

Table 5. Diagnostic characteristics of Samples One and Two

Diagnosis	Sample 1 (n = 54) N	%	Sample 2 (n = 46) N	%	Analysis χ^2	p
Attention Deficit Hyperactivity Disorder	50	92.6	41	89.1	0.36	n.s.*
Conduct Disorder	16	29.6	21	45.7	2.74	n.s.
Oppositional Defiant Disorder	25	46.3	19	41.3	0.25	n.s.
Any Mood Disorder	6	11.1	6	13.3	0.77	n.s.
Major Depressive Disorder	3	5.6	6	13.3	1.70	n.s.
Dysthymic Disorder	3	5.6	0	0.0	2.58	n.s.
Any Anxiety Disorder	17	31.5	16	34.8	0.12	n.s.
Generalized Anxiety Disorder	5	9.4	7	15.2	0.84	n.s.
Obsessive-Compulsive Disorder	1	1.9	3	6.5	1.41	n.s.
Social Phobia	1	1.9	9	19.6	8.43	0.003
Separation Anxiety Disorder	15	27.8	9	19.6	0.92	n.s.

* n.s. = not significant, $p > 0.10$.

Questionnaire (CTQ) (Goyette et al., 1978). These items were selected specifically for their ability to discriminate between inattentive/overactive and aggressive children. As presented in Table 2, boys in Sample 2 had more somatic complaints by parent report than boys in Sample 1, although this difference just missed significance ($p = 0.054$).

Aggression Measures. Data regarding aggressive behavior were gathered from parents, teachers, and children. Parental report of aggression was gathered from responses to the Disruptive Disorders Module of the DISC. Teachers of the boys were interviewed over the telephone using a separate form of the Disruptive Disorders Module of the DISC (Shaffer et al., 1989). Parents and teachers also rated the frequency and severity of various types of aggressive behavior in the past six months using separate

forms of the Children's Aggression Scale (CAS) (McKay et al., 1993). The CAS was developed specifically to evaluate verbal aggression, aggression toward objects or animals, physical aggression towards people, and the use of weapons by children. Moreover, the parent version of the CAS distinguishes between aggression inside and outside of the home, and against children versus adults. The presence or absence of aggressive symptoms was ascertained from the boys in Sample 1 using the Disruptive Disorders Module of the child DISC and from the boys in Sample 2 using the Self-Report of Antisocial Behavior Scale (Loeber et al., 1989). The Self-Report of Antisocial Behavior Scale is a semi-structured interview designed for use with elementary school children. The scale assesses a wide range of aggressive and non-aggressive antisocial acts, and determines whether these behaviors occurred "never," "once or twice," or "more often" during the past six months. As shown in Table 3, parent and teacher ratings of aggression differed between aggressive and nonaggressive boys, but did not differ across the samples. The only exceptions were parent ratings of aggression against objects and animals on the CAS, which did not differ between aggressive and nonaggressive boys, and teacher ratings of provoked physical aggression on the CAS, which differed across the samples.

Cognitive Measures. General cognitive abilities were assessed to rule out the presence of mental retardation. However, the assessment measures varied slightly between the two samples. The boys in Sample 1 were evaluated with the Wechsler Intelligence Scale for Children-Revised (WISC-R) (Wechsler, 1974), while the boys in Sample 2 were assessed with the updated Wechsler Intelligence Scale for Children-Third Edition (WISC-III) (The Psychological Corporation, 1991). Both the WISC-R and the

WISC-III yield separate Verbal, Performance and Full Scale IQ scores. As shown in Table 2, boys in Sample 1 had a higher Full Scale IQ on the WISC-R than boys in Sample 2 did on the WISC-III. This difference is likely to be attributable to differences between the WISC-III and the WISC-R, and not differences in the sample. Children score on average 5 points lower on the WISC-III than the WISC-R (The Psychological Corporation, 1991).

Demographic Interview. A semi-structured interview designed to obtain demographic information about the family was administered to the parents. The interview queried about the educational level and occupation of the boys' primary caretakers. Parental responses to the demographics interview were used to calculate socioeconomic status according to Hollinghead's four-factor method (Hollingshead, 1975). This method uses parents' or adult guardians' marital status, occupation, and educational level to generate a score that ranges from eight to 66, with 66 representing the highest social class and eight the lowest social class. Socioeconomic status did not differ between the two samples of boys, as shown in Table 2.

Family History Interview. The family history of each boy was assessed for psychiatric and behavioral symptomatology using a semi-structured interview administered to the boy's parents. The respondents initially completed a genogram diagraming the boy's first- and second-degree relatives. The genogram was then used as a guide while the interviewer systematically asked about problems in a variety of symptom domains that each family member may have experienced both as a child (where appropriate) and as an adult. Because the interview was conducted with someone other than the index person (family history method), not all criteria for each condition could be

reliably ascertained. Rather, the interview attempted to identify problem behaviors within recognizable domains of function. Problem areas assessed included aggressive behavior (i.e., fighting, causing injury to others, use of weapons, destruction of property), antisocial behavior (i.e., stealing, fire-setting, trouble with the law), symptoms of ADHD (i.e., inattention, fidgetiness), cognitive impairment (i.e., learning problems, slower than others, history of special education), and internalizing symptoms (i.e., excessive worrying, depression). When a positive report was elicited for any of these conditions, additional probes were used to determine whether the episode represented a persistent pattern of behavior or caused functional impairment. Report of impairment or recurrent behavior in any one symptom domain was considered to be indicative of a positive history for that relative.

Family history data were obtained for parents, grandparents, aunts, and uncles who were full biological relatives of the probands. Siblings were not systematically assessed because the overwhelming majority were either half-siblings or were below the ages of risk for the domains assessed. Since the remaining relatives were all over 18 years-of-age and beyond the age of risk for ADHD (i.e., 7 years), cognitive impairment (i.e., 15 years), and aggressive and antisocial behavior (i.e., 18 years), the observed rates of these symptoms in relatives did not need to be age-corrected. However, many of the relatives were still within the age of risk for internalizing symptoms (i.e., 15 to 59 years). Unfortunately, constraints imposed by the family history method limited the collection of data on the age of relatives, which precluded the age-correction of the rate of internalizing symptoms. Thus, the rate of internalizing symptoms among relatives is likely to be underestimated. The boys in Sample 1 had a significantly greater rate of

Table 6. Mean percentage of first and second-degree relatives of boys in Samples One and Two with lifetime histories of psychiatric and behavioral symptomatology

	Sample 1	Sample 2	Analysis	
Symptom Domain	%	%	t	*p*
Aggression	22.8	23.2	0.08	n.s.*
Antisocial Behavior	16.7	20.3	0.88	n.s.
Inattention/Hyperactivity	13.8	14.0	0.05	n.s.
Cognitive/Learning	12.7	9.2	1.42	n.s.
Internalizing	17.6	7.8	3.07	0.003

* n.s. = not significant, $p > 0.10$.

internalizing symptoms among relatives than the boys in Sample 2 (Table 6).

Reliability of the distinct subscales of the family history interview was determined with α coefficients. However, reliability could only be determined for data on parents and grandparents, because of constraints imposed by the collection of data on aunts and uncles. Internal consistency of the subscales was variable. Assessment of family history of aggressive ($\alpha = 0.72$) and antisocial ($\alpha = 0.70$) behaviors, symptoms of ADHD ($\alpha = 0.62$), and internalizing symptoms ($\alpha = 0.58$) had adequate reliability. Reliability of the assessment of cognitive impairment was weak ($\alpha = 0.44$).

Fenfluramine Challenge Procedure. The fenfluramine challenge procedure is based on a body of research that indicates 5-HT has a disinhibitory influence on the secretion of the anterior pituitary hormone prolactin (reviewed in Cowen et al., 1990). The secretion of prolaction into the circulatory system is induced by prolactin releasing factor (PRF) (Laudon et al., 1990). PRF is synthesized by parvocellular neurons in the paraventricular nucleus (PVN) of the hypothalamus and released into the median

eminence (Rittenhouse et al., 1993), where it is transported through the hypophysial portal system to the anterior pituitary (Laudon et al., 1990). The release of PRF, and consequently prolactin, is tonically inhibited by neurons of the tuberoinfundibulary dopamine system (Fuller, 1992). These dopamine neurons, in turn, are phasically inhibited by ascending 5-HT fibers of the dorsal raphe nucleus via postsynaptic 5-$HT_{2A/2C}$ receptors (Jacobs & Azmitia, 1992). Thus, increased raphe 5-HT activity inhibits dopamine activity in the tuberoinfundibulary system, which disinhibits prolactin release.

Fenfluramine is a known sympathomimetic agent that releases endogenous stores of 5-HT, blocks reuptake of synaptic 5-HT, and stimulates postsynaptic 5-HT receptors both directly and indirectly (Rowland & Carlton, 1986). The resultant increase in synaptic levels of 5-HT in the hypothalamic PVN produces a robust rise in plasma prolactin levels that is dose-dependent (Lewis & Sherman, 1985), reliable over periods of up to two years (Pick et al., 1999), and can be blocked by 5-$HT_{2A/2C}$ receptor antagonists (Coccaro et al., 1996c). The magnitude of the prolactin response to fenfluramine challenge provides a dynamic measure of combined pre- and postsynaptic 5-HT function in the hypothalamic-pituitary axis (Fuller, 1992). While the racemic isomer of fenfluramine used in the current studies also influences dopamine transmission, these effects have only been reported with substantially higher doses than used presently (Meyerdorff et al., 1986). In fact, prolactin responses to the dose of *d,l*-fenfluramine used in the current study are highly correlated with prolactin response to comparable doses of the more 5-HT-selective *d*-fenfluramine in adults (Coccaro et al., 1996d).

The fenfluramine challenge was conducted in the Mount Sinai Clinical Research Center on one day approximately one week following the clinical evaluation.

Participants were medication-free for at least four weeks prior to the fenfluramine challenge and were maintained on a low MAO diet for at least three days prior to the study. The protocol began after an overnight fast, at 8:00 am, with the insertion of an indwelling intravenous catheter (kept open with normal saline) into a forearm vein. Samples of blood for baseline plasma prolactin and cortisol were obtained at 9:45 am (-15 min.) and at 9:55 am (-5 min.). At 10:00 am (0 min.), 1 mg/kg of *d,l*-fenfluramine was administered orally. Post-fenfluramine samples of plasma prolactin, cortisol, fenfluramine, and its active metabolite norfenfluramine were obtained hourly until 3:00 pm. Additionally, vital signs were checked every half hour. The children remained awake and fasting during the entire procedure, watching G-rated videotaped movies. The protocol ended at 3:00 pm, at which time the catheter was removed and the boys were given a meal.

Blood samples were placed immediately on ice until centrifugation, which took place within two hours. After separation, blood samples for prolactin assay remained frozen at -80°C until assayed by radioimmunoassay (Kahn et al., 1994). The lower limit of detection for the prolactin assay was <1.0 ng/ml. Intra- and inter-assay variability were less than 6.7% and 8.4%, respectively. The sensitivity of the cortisol assay was 0.5 μg/ml% and the ED_{50} is 8.2 μg/ml%. The intra- and inter-assay variability of the cortisol assay were less than 6% and 10%, respectively. Samples for plasma fenfluramine and norfenfluramine levels were collected in a borosilicate acid-washed glass tube with balanced ammonium-potassium oxylate crystals as the anticoagulant. After separation, plasma was frozen at -20°C until assay by gas-chromatography with

electrical detection (Krebs et al., 1984). The lower limit of sensitivity was 2 ng/ml for fenfluramine and 3 ng/ml for norfenfluramine. Intra- and inter-assay variability were less than 7% for both fenfluramine and norfenfluramine.

The value peak delta prolactin was computed by subtracting the mean of the two baseline prolactin samples from the peak prolactin level following fenfluramine administration. Peak delta prolactin served as the measure of the prolactin response to fenfluramine in the three studies of this dissertation. Peak delta prolactin is highly correlated with other measures typically used to assess prolactin response to fenfluramine, such as prolactin area under the curve. Thus, separate analyses were not warranted. Medication level was calculated as the sum of the fenfluramine and norfenfluramine levels at the time of peak prolactin. As shown in Table 2, peak delta prolactin values did not differ across Samples 1 and 2.

STUDY ONE:

Lack of Seasonal Rhythms in Central 5-HT Function in Boys with ADHD

Recent studies suggest that some of the variability among the child studies of central 5-HT may be accounted for by extraneous factors, such as seasonal variations in indices of 5-HT function. Circannual seasonal variations have been consistently described in platelet 5-HT uptake and [^{3}H]paroxetine binding, hypothalamic 5-HT concentrations, plasma levels of tryptophan and 5-HT, CSF concentrations of 5-HIAA, and neuroendocrine responses to challenge with 5-HT agonists in healthy adults (Brewerton et al., 1988; D'Hondt et al., 1996; Klompenhouwer et al., 1990; Maes et al., 1995; Monteleone et al., 1999; Sarrias et al., 1989) and adult patients with depression (Arora et al., 1984; Cappiello et al., 1996; Carlsson et al., 1980), bulimia (Brewerton et al., 1992), and obsessive-compulsive disorder (Grossman et al., 1996). While not entirely consistent, these studies suggest that adults may have elevated 5-HT function in the late Summer and Fall, with reduced 5-HT activity in the Spring. This seasonality in 5-HT has been linked to such weather variables as ambient temperature, relative humidity, and air pressure (D'Hondt et al., 1996). Despite the considerable interest in the relationship between 5-HT and aggression, however, seasonality in 5-HT measures has only been studied in relation to attempted violent suicide (Maes et al., 1995), but not other types of aggressive behaviors.

Whereas the data suggest seasonal rhythms in 5-HT activity in adults, similar evidence in children is scant and inconsistent. One study found a peak in platelet [^{3}H]imipramine binding during the summer in adolescent suicide attempters, but not in

nonsuicidal adolescents with conduct disorder (Pine et al., 1995). However, this same research group later reported a trough in platelet 5-HT_{2A} receptor density during the same months in a non-referred sample of boys at risk for antisocial behavior (Pine et al., 1996). In contrast, no seasonal variations were identified in CSF 5-HIAA concentrations in a heterogeneous sample of children with DBD (Swedo et al., 1989).

Taken together, the discrepant findings among similar populations of at-risk children suggests that seasonal rhythms in 5-HT function may vary as a function of the biological measure studied.. Conversely, the finding of seasonal differences in the same measure of 5-HT (i.e., [^{3}H]imipramine binding) between diagnostic groups of children raises the possibility that 5-HT seasonality may also vary as a function of psychiatric status. The latter hypothesis might account for some of the variability among the studies of central 5-HT and childhood aggression performed in this laboratory (Halperin et al., 1994, 1997a). Thus, the aim of the current study was to determine whether distinct seasonal fluctuations in central 5-HT activity exist among aggressive and nonaggressive boys with ADHD. It was predicted that aggressive boys would exhibit either a larger summertime and autumnal peak or a smaller springtime trough in central 5-HT activity than nonaggressive boys, and that these seasonal effects would mask the inverse relationship between central 5-HT activity and aggression in the sample.

SPECIFIC METHODS.

Subjects. The participants were 91 boys from Samples 1 and 2 who met DSM-III-R criteria for ADHD or DSM-IV criteria for ADHD, combined type, respectively.

The sample in this study was limited to boys with ADHD to address the possibility that 5-HT seasonality may vary as a function of psychiatric status. The boys were divided into aggressive (n = 51) and nonaggressive (n = 40) groups using the aforementioned procedures. As can be seen in Table 7, dates of sampling were relatively uniformly distributed across the four seasons and did not differ for the aggressive and nonaggressive groups (χ^2 = 1.82, df =3, $p > 0.10$). This study was conducted in New York City, which at 40.40° N latitude experiences substantial climatic change across the seasons.

Data analysis. Psychometric characteristics of the aggressive and nonaggressive boys with ADHD were compared using Student's t-tests and Pearson's chi-square analyses. To control for potential differences in fenfluramine metabolism, the dependent variable of peak delta prolactin was corrected for medication level at the time of peak prolactin using linear regression. These residualized peak delta prolactin values were analyzed for seasonal rhythms using the cosinor analysis method (Bingham et al., 1982; Cappiello et al., 1996). This method permits the detection of circannual rhythms and

Table 7. Seasonal distribution of sampling dates for aggressive and nonaggressive boys with ADHD

	Nonaggressive	Aggressive	
Season	*n*	*n*	Total
Winter	12	12	24
Spring	11	13	24
Summer	7	15	22
Autumn	10	11	21

derivatives thereof (e.g., 26 weeks) in biological measures using single samples. The prolactin values were collected over the course of an 8-year period (1990-1997), but were collapsed across years and analyzed in terms of day of the calendar year (i.e., 1 thru 365). In addition to an annual period, periods of 6 months, 3 months, 2 months, 1 month, and one-half month were included in the analysis, to fit a 12 parameter model including a phase parameter for each period. This permits considerable flexibility which allows for detection of irregularly spaced peaks and troughs. Stepwise linear regression was used to examine the interaction of seasonality with aggression. The first step consisted of the 12 seasonality variables. The second step consisted of the dichotomous aggression variable, which was entered as a prelude to the examination of the interaction of seasonality with aggression. The third step consisted of 12 interaction predictors, each the product of the dichotomous aggression variable with one of the seasonality variables. The F-test of the change in R^2 for the third step was used to test the hypotheses of differences in 5-HT seasonality among aggressive and nonaggressive boys. The α level for these analyses was set at the 0.05 level, and all probabilities are based on two-tailed tests.

RESULTS

Psychometric and diagnostic characteristics. As shown in Table 8, the aggressive and non-aggressive boys with ADHD did not differ in age, SES, or ratings of hyperactivity, but did differ in Full Scale IQ and parent and teacher ratings of aggression. As expected, the aggressive boys also had a significantly greater rate of conduct disorder

(58.8% versus 7.5%;$\chi^2 = 25.55$, df = 1, $p < 0.001$) than the non-aggressive boys. The rate of anxiety did not differ between the groups ($p > 0.10$), but aggressive boys did have a greater prevalence of mood disorders than nonaggressive boys (15.7% versus 2.6%; $\chi^2 = 4.23$, df = 1, $p = 0.04$).

Seasonality in central 5-HT function. As shown in Figure 4, there was no evidence of distinct annual, biannual, 3-monthly, 2-monthly, monthly, or bimonthly rhythms in peak delta prolactin among aggressive and nonaggressive boys with ADHD. More specifically, stepwise linear regression revealed that peak delta prolactin in the entire sample did not fit to a sinusoidal function, with the 12 combined seasonality variables entered in step one accounting for only a small proportion of the variance (R^2 =

Table 8. Demographic and psychometric characteristics of aggressive and nonaggressive boys with attention-deficit/hyperactivity disorder (ADHD)

	Nonaggressive (n = 40)		Aggressive (n = 51)		Analysis	
Variable	Mean	S.D.	Mean	S.D.	t	p
Age (in years)	9.1	1.3	9.1	1.3	0.36	n.s.*
Socioeconomic Status	34.1	14.9	29.3	14.6	1.53	n.s.
Full Scale IQ	97.9	12.6	92.7	12.5	2.08	0.04
Child Behavior Checklist						
Attention Problems	72.0	9.7	72.7	10.4	0.34	n.s.
Aggression	63.0	11.0	77.7	12.8	5.47	<0.001
IOWA Conners						
Inattention/Overactivity	11.1	2.8	11.5	2.9	0.63	n.s.
Aggression/Defiance	6.0	4.6	9.7	3.7	4.06	<0.001

* n.s. = not significant, $p > 0.10$.

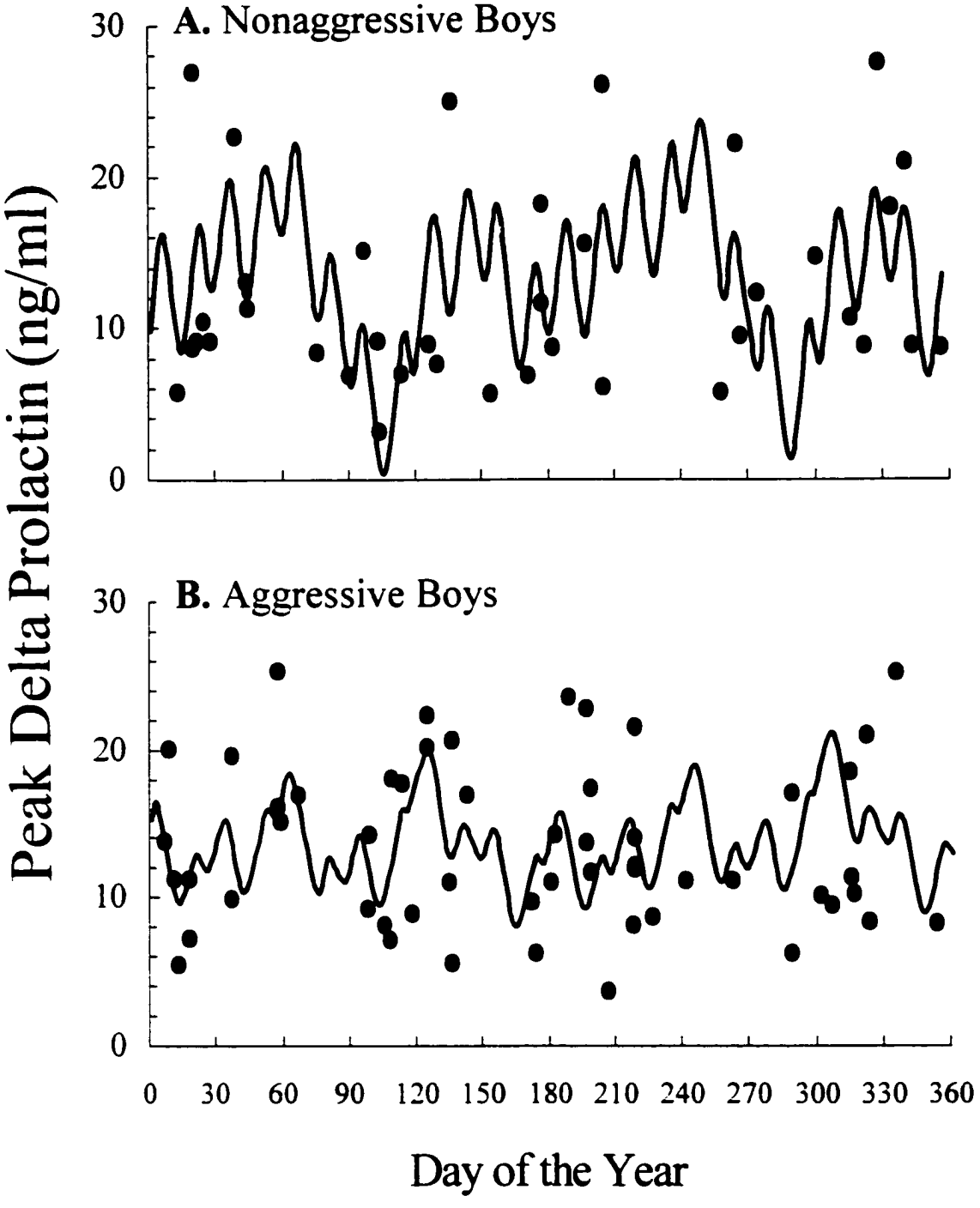

Figure 4. Lack of Seasonal Rhythms in Peak Delta Prolactin Response to *d,l*-fenfluramine in A. Nonaggresive (n=40) and B. Aggressive (n=51) Boys with ADHD. Unresidualized prolactin values are plotted to simplify presentation.

0.12. F = 0.88, df = 12,78, p = 0.57). The regression analysis also revealed no evidence of an interaction between seasonality and aggression. The dichotomous aggression-group variable, entered in step two as a preliminary to examination of the interaction of seasonality with aggression, was not related to peak delta prolactin (R^2 = 0.004, F = 0.33, df = 1,77, p = 0.57). More importantly, the 12 seasonality-by-aggression group interaction predictors entered in step three of the regression did not account for a significant proportion of additional variance in peak delta prolactin (R^2 = 0.08, F = 0.52, df = 12,65, p = 0.90). Statistically controlling for group differences in Full Scale IQ or the prevalence of mood disorders did not significantly change the results.

DISCUSSION

This study found no evidence of seasonal variations in central 5-HT function in a relatively large sample of prepubertal boys with ADHD. More importantly, there was no indication of group-specific seasonal differences in central 5-HT function between aggressive and nonaggressive boys with ADHD. Specifically, cosinor analysis revealed no annual, biannual, 3-monthly, 2-monthly, monthly, and bimonthly rhythms in the prolactin response to fenfluramine in the entire sample of boys or in the separate aggressive and nonaggressive groups. These results are consistent with those from a previous study that failed to detect seasonal variations in CSF 5-HIAA concentrations in children with DBD, many of whom had ADHD (Swedo et al., 1989). The fact that similar results have now been found using both CSF and neuroendocrine challenge measures of central 5-HT activity suggests that this lack of seasonality in boys with

ADHD is not simply an artifact of sampling methods.

The absence of group-specific seasonal differences in central 5-HT activity indicates that seasonality-related variance does not account for the considerable overlap among the aggressive and nonaggressive boys in the current sample. These results suggest that further analyses of the relationship of central 5-HT activity to aggression in the current sample need not control for seasonal variations. However, the current findings must be considered in the context of two methodological limitations. First, the data were derived from different children over the course of eight years, rather than from repeated sampling of the same children throughout the year. Therefore, seasonal fluctuations in central 5-HT function might have been masked by differences among the children in some unmeasured characteristics (e.g., impulsiveness). Nevertheless, this methodological weakness is partly balanced by the relatively large sample size (N = 91). Second, the limited set of frequencies examined in this study may have failed to detect a significant rhythm in the prolactin response, although visual inspection of the data (Figure 3) suggests that this is not very likely.

The present analyses cast considerable doubt on the hypothesis that seasonality-related variance accounts for the discrepancy among the child studies of central 5-HT. Rather, the inconsistencies are likely due to such intrinsic factors as differences in samples. Attempts to reconcile the inconsistencies across the child studies of central 5-HT have noted sample differences, such as in age and the proportion of subjects with ADHD (Castellanos et al., 1994; Halperin et al., 1997a; Pine et al., 1997).

STUDY TWO:

The Impact of Age and ADHD on the Relationship Between Central 5-HT Function and Aggression in Prepubertal Boys

Attempts by several investigators to reconcile the differences across the child studies of central 5-HT function and aggression indicated that the samples differed in age and the proportion of subjects with ADHD (Castellanos et al., 1994; Halperin et al., 1997a; Pine et al., 1997). Among the six studies, central 5-HT function was positively correlated with aggression in the three youngest samples (Castellanos et al., 1994; Halperin et al., 1994; Pine et al., 1997), not associated with aggression in the two slightly older prepubertal and adolescent samples (Halperin et al., 1997a; Stoff et al., 1992), and inversely related to aggression in the oldest sample (Kruesi et al., 1990, 1992). These findings raise the possibility that a developmental downregulation in central 5-HT activity may explain the discrepancy among the studies of 5-HT and childhood aggression, as well as provide a link between the child and adult literature. Non-linear developmental changes in 5-HT receptor binding have been described in rats (Whitaker-Azmitia et al., 1990), monkeys (Lidow et al., 1991), and humans (Biegon and Greuner, 1992), such that enhanced binding in early childhood is followed by a decline through puberty. Several studies have also found CSF 5-HIAA to be inversely correlated with age in boys (Hyland et al., 1994; Kruesi et al., 1990). Moreover, one recent study reported a developmental decrease in the prolactin response to fenfluramine over a two-year period in a subgroup of a larger sample of boys with ADHD (Pick et al., 1999).

Another possible explanation for the inconsistent findings posits differences in

comorbid disorders across samples, specifically in the proportion of subjects with ADHD. Among the three studies that reported a positive correlation between central 5-HT function and aggression, two were selected specifically for ADHD (Castellanos et al., 1994; Halperin et al., 1994), and the third consisted of a non-referred sample with a large number of boys who met criteria for ADHD (Pine et al., 1997). In contrast, the two studies that reported an inverse or no association between 5-HT and aggression were conducted in more heterogenous samples of boys with DBD that contained fewer cases of ADHD (Kruesi et al., 1990, 1992; Stoff et al., 1992). Thus, the presence of ADHD may influence the relationship between central 5-HT function and aggression in children. While ADHD is generally associated with catecholaminergic dysfunction (Zametkin and Rapoport, 1987), central dopaminergic and 5-HT systems are highly interrelated in humans (Geracioti et al., 1998; Hill et al., 1999). Consequently, perturbations in dopamine activity associated with the presence of ADHD could alter central 5-HT function in children, thereby modifying the relationship between this variable and aggression. The hypothesis that 5-HT function varies with the presence or absence of ADHD could also help resolve the apparent inconsistencies between the child and adult literature. Symptoms of ADHD decrease with age in many individuals, even in some who remain symptomatic (Weiss et al., 1985), and these longitudinal changes in symptomatology may be mediated in part by developmental changes in central 5-HT function (Biegon and Greuner, 1992; Lidow et al., 1991; Whitaker-Azmitia et al., 1990). Thus, it may be that early aggression is present in a heterogeneous group of children, many of whom will not continue to exhibit aggression, and that this latter group is not characterized by alterations in central 5-HT function.

This study examined whether age or the presence of ADHD influenced the relationship between central 5-HT function and aggression in nonaggressive boys with ADHD, aggressive boys with ADHD, and aggressive boys without ADHD. Ethical and practical considerations prevented the inclusion of a group of nonaggressive boys without ADHD in this study. It was hypothesized that central 5-HT responsivity would be: a) lowest in aggressive boys without ADHD and highest in aggressive boys with ADHD, with nonaggressive boys with ADHD falling in between; and b) greater in young aggressive boys relative to young nonaggressive boys, but would fail to differ among older boys.

SPECIFIC METHODS.

Subjects. The 46 boys in Sample 2 were divided into nonaggressive ADHD, aggressive ADHD, and aggressive non-ADHD groups using the aforementioned procedures. This study was limited to boys in Sample 2, because the sample had been recruited specifically to examine the effect of ADHD on central 5-HT function and included a group of aggressive boys without ADHD for comparison. In contrast, the boys in Sample 1 had all been recruited for the presence of ADHD. The final groups consisted of 13 nonaggressive boys with ADHD, 28 aggressive boys with ADHD, and 5 aggressive boys without ADHD. As was the case for Klein et al. (1997), most boys referred for aggressive behavior also met criteria for ADHD according to the DISC.

Data analysis. Psychometric and diagnostic characteristics of the nonaggressive ADHD, aggressive ADHD, and aggressive non-ADHD groups were compared using one-

way analyses of variance (ANOVA) and chi-square analyses, respectively. The primary tests of the apriori hypotheses were conducted with analyses of covariance (ANCOVA) controlling for medication level. The impact of ADHD on the association between central 5-HT and aggression was tested using a one-way ANCOVA to evaluate differences in peak delta prolactin across the three groups of boys. A separate two-way ANCOVA with age-group and the presence or absence of aggression as the between-group factors was used to analyze the effect of age on the relationship between 5-HT and aggression. Age-group was determined via a median split of the entire sample ($N = 46$) on age. Bonferroni's correction was used to control for the inflated α level resulting from the use of multiple ANCOVAs. Thus, a p value of 0.025 (i.e., 0.05 / 2) was used as the cutoff for significance for the two ANCOVAs.

Secondary analyses examined the dimensional relationship between peak delta prolactin and age, measures of ADHD, and ratings of aggression using Pearson's product-moment correlations. Further, to remove the potential impact of ADHD, the association between peak delta prolactin and ratings of aggression was analyzed again using partial correlations controlling additionally for ratings of ADHD. Due to the large number of correlations performed, the α level for these analyses was set at the 0.01 level to avoid type I errors. All probabilities are based on two-tailed tests.

RESULTS

Demographic characteristics. Basic demographic and psychometric

Table 9. Demographic and psychometric characteristics of nonaggressive boys with ADHD, aggressive boys with ADHD, and aggressive boys without ADHD

	Nonaggressive ADHD (n = 13)		Aggressive ADHD (n = 28)		Aggressive non-ADHD (n = 5)		Analysis	
Variable	Mean	S.D.	Mean	S.D.	Mean	S.D.	F	p
Age (in years)	9.3	1.5	9.3	1.2	10.4	0.7	1.68	n.s.*
Socioeconomic Status	34.4	11.8	28.9	15.8	26.1	9.3	0.83	n.s.
Full Scale IQ	96.7	11.9	92.2	12.7	84.2	9.9	1.92	n.s.
Child Behavior Checklist								
Attention Problems	75.1	11.7	72.7	11.8	70.8	7.8	0.37	n.s.
Aggression	64.7	12.2	79.5	12.4	71.2	11.1	6.71	<0.01
IOWA Conners								
Inattention/Overactivity	12.3	2.5	11.6	2.8	9.4	5.0	1.65	n.s.
Aggression/Defiance	6.9	5.7	9.6	3.7	11.2	2.3	2.37	n.s.

* n.s. = not significant, $p > 0.10$.

characteristics of the nonaggressive ADHD, aggressive ADHD, and aggressive non-ADHD groups are presented in Table 9. As shown, the groups differed significantly in parent ratings of aggression, but not in teacher ratings on the Aggression/Defiance scale of the IOWA. The aggressive ADHD and aggressive non-ADHD groups had a greater rate of conduct disorder than the non-aggressive ADHD group (61% and 40% versus 15%; $\chi^2 = 7.43$, df = 2, p = 0.02). There was no difference in the rate of mood and anxiety disorders among the groups (all $p > 0.10$).

Central 5-HT activity, aggression, and ADHD. As depicted in Figure 5, the one-way ANCOVA assessing peak delta prolactin revealed no significant difference across the non-aggressive ADHD, aggressive ADHD, and aggressive non-ADHD groups (F = 0.06, df = 2,42, p = 0.94). When examined dimensionally, Pearson product-moment

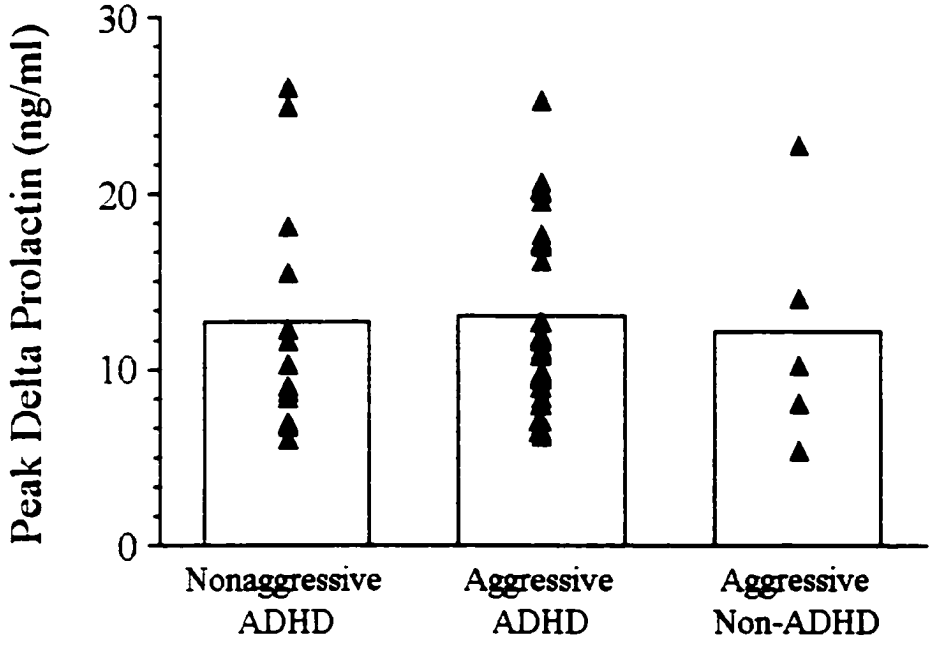

Figure 5. Mean peak delta prolactin response to *d,l*-fenfluramine in nonaggressive boys with ADHD, aggressive boys with ADHD, and aggressive boys without ADHD (One-way ANCOVA: F = 0.30, df = 2,38, $p>0.10$).

correlations revealed no significant association between peak delta prolactin and ratings of aggression. Furthermore, as shown in Table 10, correlations between peak delta prolactin and parent and teacher ratings of aggression were largely unaffected when ratings of ADHD were entered as a covariate. Moreover, there was no relationship between peak delta prolactin and ADHD symptom count ($r = -0.07$, $df = 46$, $p = 0.65$), parent ratings on the CBCL Attention factor ($r = 0.05$, $df = 46$, $p = 0.73$), or teacher

Table 10. Pearson correlations and partial correlations controlling for ADHD between peak delta prolactin and ratings of aggression in boys (N=46)

Instrument	Pearson Correlations		Partial Correlations controlling for ADHD	
	r	*p*	r	*p*
Child Behavior Checklist[a]				
Aggression	0.06	n.s.[b]	0.04	n.s.
Children's Aggression Scale - Parent[a]				
Verbal aggression	0.04	n.s.	0.01	n.s.
Aggression against objects & animals	0.02	n.s.	-0.01	n.s.
Provoked physical aggression	0.04	n.s.	0.02	n.s.
Initiated physical aggression	0.08	n.s.	0.07	n.s.
IOWA Conners[c]				
Aggression/Defiance	0.22	n.s.	0.18	n.s.
Children's Aggression Scale - Teacher[c]				
Verbal aggression	0.27	n.s.	0.24	n.s.
Aggression against objects & animals	0.38	0.01	0.35	n.s.
Provoked physical aggression	0.11	n.s.	0.09	n.s.
Initiated physical aggression	0.21	n.s.	0.19	n.s.

[a] Partial correlations controlling for Child Behavior Checklist Attention Problems score.

[b] n.s. = not significant, $p > 0.10$.

[c] Partial correlations controlling for IOWA Conners Inattention/Overactivity score

ratings on the IOWA Inattention/Overactivity score ($r = 0.15$, $df = 43$, $p = 0.34$).

Central 5-HT activity, aggression, and age. Two-way ANCOVA assessing differences in peak delta prolactin yielded no significant main effects for age-group ($F = 0.45$, $df = 1,41$, $p > 0.51$) or aggression ($F = 0.01$, $df = 1,41$, $p = 0.99$), and no age-group-by-aggression interaction ($F = 0.06$, $df = 1,41$, $p = 0.81$). Finally, there was no significant dimensional relationship between peak delta prolactin and age ($r = 0.03$, $df = 46$, $p = 0.84$).

DISCUSSION

These data represent the largest independent sample of children to date in which the association between central 5-HT function and aggression has been examined. The failure to find evidence of an association between central 5-HT activity and aggression in the current study are in agreement with the results from a previous sample (Halperin et al., 1997a), and suggest that there is no straight forward relationship between central 5-HT and aggression in prepubertal boys. Moreover, these data do not confirm the hypothesis that age or the presence of ADHD influence the relationship between 5-HT and childhood aggression. Specifically, the prolactin response did not differ among nonaggressive boys with ADHD, aggressive boys with ADHD, and aggressive boys without ADHD. Moreover, this negative finding is not due to a lack of power from the small sample size in the aggressive non-ADHD group. The mean prolactin response to fenfluramine in this group was nearly identical to that of the other two groups (effect size = 0.05). Thus, even if the sample size was increased by ten-fold, with this effect size, the

difference would not have been significant. The prolactin response was also largely unrelated to dimensional measures of aggression, even after the potential impact of ADHD was statistically removed. Finally, in contrast to our previous report (Halperin et al., 1997a), the relationship between the prolactin response and aggression did not vary as a function of age in the current study. Taken together, these data tend to discount age or the presence of ADHD as possible explanations for the discrepancy among the studies of 5-HT and childhood aggression. However, the findings of the current study must be considered in the context of the fact that the small number of aggressive children without ADHD in this study may not be representative of aggressive non-ADHD children in the population.

The source of the discrepancies across the studies of central 5-HT activity and child aggression continues to remain elusive. The results of Study 1 seemed to rule out seasonal variation in central 5-HT as a possible explanation for the variability, at least in the current sample. Moreover, the results of the current analyses also cast considerable doubt on both age and diagnostic comorbidity as possible explanations for the inconsistency among the child studies of central 5-HT, as had been suggested by several of the investigators (Castellanos et al., 1994; Halperin et al., 1997a; Pine et al., 1997). However, these investigators also noted considerable variability in the severity of aggression across samples (Castellanos et al., 1994; Halperin et al., 1997a), and raised the possibility that the inconsistency of neurochemical findings in boys may be attributable to the heterogeneous nature of aggressive behavior in young boys. Only a small proportion of aggressive boys become aggressive adults (Loeber et al., 1993). It is likely that the numerous children who are classified as aggressive, but who are likely to

desist in their aggression later in life, may not have the same neurobiology as those children whose aggression is likely to progress.

Study Three:

Familial Correlates of Central 5-HT Function in Prepubertal Boys

The heterogeneous nature of aggressive behavior in children may also account for the variability across the child studies of central 5-HT function. Many young children engage in some aggressive behavior, but only a small proportion of aggressive children progress to physical fighting as adolescents, and an even smaller number escalate to serious violence as adults (Loeber et al., 1993). Social, familial, peer, and individual factors all play a role in the development and progression of aggressive behavior in children (reviewed in Farrington & Loeber, 2000). Consequently, it is possible that the contribution of central 5-HT dysfunction to childhood aggression may be obscured by the numerous reasons children become aggressive. Many of these children will desist in their aggression later in life, and may not have the same neurobiology as those children whose aggression is likely to escalate.

Several lines of research have identified familial sociopathy and aggression as major risk factors for the progression of childhood aggression into adolescent and adult violence and criminality (Cadoret et al., 1995; Frick et al., 1992). It may also be that familial aggression and sociopathy are associated with reduced central 5-HT function. Studies in child and adult samples have reported low CSF 5-HIAA concentrations in individuals with high rates of familial criminality and APD (Constantino et al., 1997; Virkunnen et al., 1996). Further, a blunted prolactin response to fenfluramine has been reported to be associated with high rates of impulsive aggression in the first degree relatives of adults with personality disorders (Coccaro et al., 1994) and with parental

history of aggression in aggressive children (Halperin et al., 1997b).

These data suggest that familial transmission of aggressive behavior may be associated with low central 5-HT function. The current study tested this hypothesis by examining the incidence of aggressive and antisocial behaviors in first and second degree relatives of aggressive and nonaggressive children who were further subdivided based upon central 5-HT responsivity. It was postulated that the aggressive children with low 5-HT function would have an higher frequency of relatives with aggressive and antisocial behaviors when compared to both nonaggressive children and aggressive children with relatively higher 5-HT activity.

SPECIFIC METHODS

Subjects. The participants included all 91 boys in Samples 1 and 2 for whom family history data were ascertained. The boys were divided into aggressive (n = 55) and nonaggressive (n = 36) groups using the aforementioned procedures. The sample was also divided on the basis of central 5-HT responsivity using a median split on peak delta prolactin. The median peak delta prolactin for the entire sample was 11.20 ng/ml. Forty-five boys were assigned to the low delta prolactin group (mean = 8.05 ng/ml, SD = 2.00) and 46 boys were assigned to the high delta prolactin group (mean = 19.59 ng/ml, SD = 8.98). The aggressive and nonaggressive children were relatively evenly distributed across the delta prolactin groups, with 25 (45%) of the 55 aggressive boys and 20 (56%) of the 36 nonaggressive boys assigned to the low delta prolactin group (χ^2 = 0.89, df = 1,

$p = 0.35$).

Family History Assessment: The family history interview was administered either to the child's mother (N = 62), the child's father (N = 3), both parents together (N = 14), or another relative with whom the child lived (N = 12). Family history data were obtained for 175 parents, 283 grandparents, and 464 aunts and uncles, for a total of 922 biological first and second degree adult relatives.

Data Analysis. To rule-out other factors that might account for differences in peak delta prolactin, demographic and diagnostic data were assessed using Pearson's chi-square analyses and two-way analyses of variance (ANOVA) with aggression group and delta prolactin group as independent variables. The α level for these analyses was set at 0.05, and all probabilities are based on two-tailed tests. The percentage of relatives who were reported to exhibit symptomatology within the five domains assessed (i.e., aggressive, antisocial, ADHD, cognitive impairment/learning, internalizing) was calculated for each child. Separate two-way ANOVAs were used to examine the percentage of relatives with symptoms within the five domains. Aggression group and delta prolactin group served as the independent variables in these analyses. Bonferroni's correction was used to control for the inflated α level resulting from the use of five ANOVAs. Thus, a p value of 0.01 (i.e., 0.05/5) was used as the cutoff for significance for the five ANOVAs. The strength of significant findings was measured by calculating odds ratios for psychiatric symptomatology in relatives as a function of aggression group and delta prolactin group.

RESULTS.

Demographic and diagnostic characteristics. Psychometic and diagnostic characteristics of the aggressive and nonaggressive boys subdivided as a function of peak delta prolactin are displayed in Table 11. As expected, both parents and teachers rated the aggressive groups as significantly more aggressive than the nonaggressive groups. Moreover, Full Scale IQ was lower among aggressive than nonaggressive boys and in boys with low peak delta prolactin compared to boys with high peak delta prolactin. However, there was no significant aggression group-by-delta prolactin group interaction for Full Scale IQ ($p = 0.65$). Aggressive boys also had a significantly greater rate of conduct disorder than nonaggressive boys (56% versus 8%; $\chi^2 = 21.44$, $df = 3$, $p < 0.0001$). Surprisingly, the greater prevalence of mood disorders found among aggressive boys in Study 1 was primarily accounted for by those boys with low delta prolactin ($\chi^2 = 9.16$, $df = 3$, $p = 0.03$). Of the 11 boys with mood disorders in the sample, 10 were classified as aggressive, and 7 of these aggressive boys had low delta prolactin.

Familial aggression, child aggression, and central 5-HT activity. The mean percentage of relatives with psychiatric symptoms in the aggressive and nonaggressive groups, subdivided as a function of peak delta prolactin, are depicted in Figure 6. Two-way ANOVAs assessing the percentage of relatives with aggressive and antisocial behaviors both yielded significant main effects for aggression group (for aggressive behaviors: $F = 17.48$, $df = 1,87$, $p < 0.0001$; for antisocial behaviors: $F = 9.87$, $df = 1,87$, $p = 0.002$), but not for delta prolactin group (both $p > 0.01$). The aggression group-by-

Table 11. Demographic and psychometric characteristics of aggressive and nonaggressive boys as a function of peak delta prolactin response to fenfluramine (ΔPRL)

	Nonaggressive				Aggressive			
	Low ΔPRL (n = 20)		High ΔPRL (n = 16)		Low ΔPRL (n = 25)		High ΔPRL (n = 30)	
Variable	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Age (in years)	9.0	1.3	9.4	1.4	9.5	1.2	9.3	1.3
Socioeconomic status	36.2	14.1	32.8	15.3	26.8	11.7	32.0	14.7
Full Scale IQ	96.6	12.1	103.4	9.4	90.8	12.0	95.2	13.4
Child Behavior Checklist								
Attention Problems	69.9	7.6	75.8	11.7	76.7	8.8	70.0	8.9
Aggression	62.4	11.0	63.8	10.4	79.8	12.0	74.8	13.1
IOWA Conners								
Inattention/Overactivity	11.2	2.7	11.0	2.9	11.2	3.4	11.8	2.8
Aggression/Defiance	6.2	4.5	5.8	4.8	10.2	3.8	10.3	3.4

[a] Significant main effect for aggression group, $p < 0.05$.

[b] Significant main effect for delta prolactin group, $p < 0.05$.

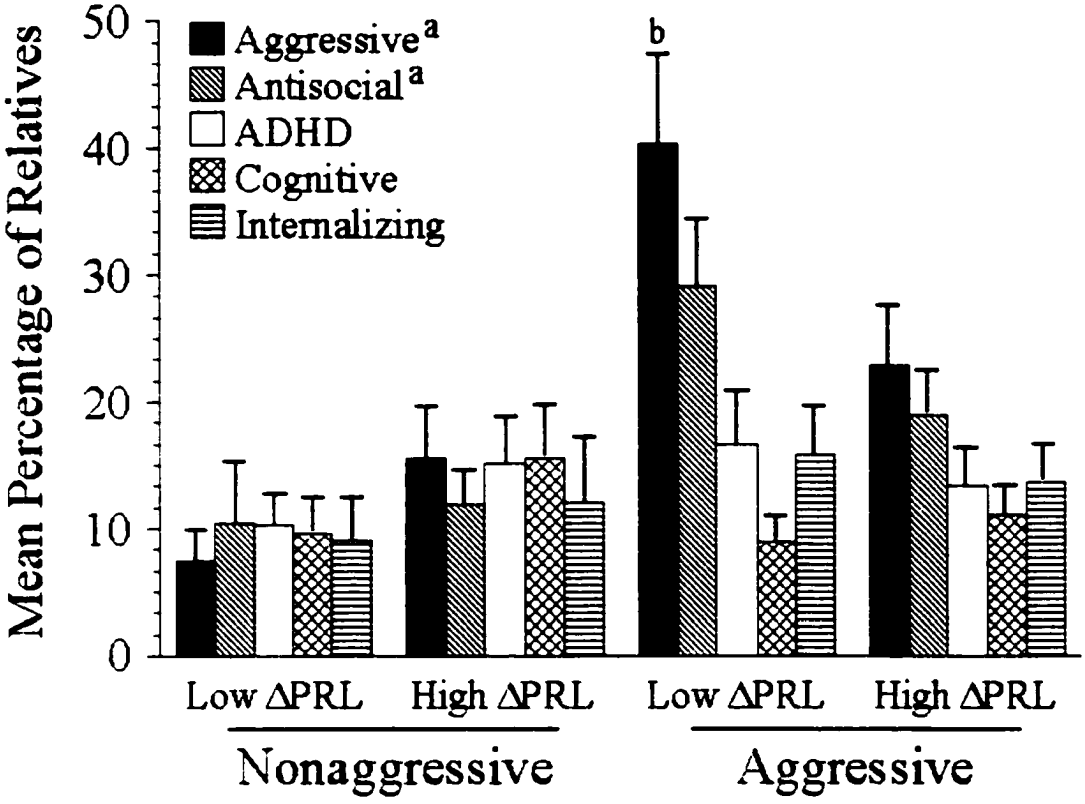

Figure 6. Mean percentage of first and second degree relatives with psychiatric symptoms as a function of child aggression and peak delta prolactin response to fenfluramine (ΔPRL).

[a] Main effect for aggression group, $p < 0.01$.

[b] Significantly greater than other three groups, $p < 0.01$.

delta prolactin group interaction was also significant for aggressive behavior in relatives ($F = 7.18$, $df = 1,87$, $p = 0.009$), but not for antisocial behavior ($F = 1.99$, $df = 1,87$, $p = 0.16$). Statistically controlling for group differences in Full Scale IQ and the rate of mood disorders did not significantly alter the results. Post hoc comparisons of the four groups using Tukey's Honestly Significant Difference tests revealed that aggressive boys in the low delta prolactin group had a significantly higher incidence of relatives with aggressive behaviors than the children in the other three subgroups. Two-way ANOVAs

assessing the percentage of relatives with positive histories for ADHD, cognitive impairment/learning difficulties, and internalizing symptoms generated no significant main effects or interactions (all $p > 0.10$).

Among the 922 relatives for whom family history data were gathered, 452 (49.1%) were male and 470 (50.9%) were female. The percentage of male and female relatives with aggressive and antisocial characteristics in the aggressive and nonaggressive groups subdivided as a function of peak delta prolactin are displayed in Figure 7. Aggressive and antisocial behaviors were significantly more common in male as compared to female relatives (for aggressive behaviors: $F = 22.41$, $df = 1,87$, $p < 0.0001$; for antisocial behaviors: $F = 27.05$, $df = 1,87$, $p < 0.0001$). However, the pattern of family history findings was virtually identical for relatives of both genders; there was no interaction of gender with aggression group and/or delta prolactin group (all $p > 0.10$). Aggressive boys in the low delta prolactin group had an increased frequency of aggressive characteristics among both male and female relatives.

Further exploratory analyses examined the incidence of aggressive and antisocial behaviors in parents, aunts and uncles, and grandparents separately using two-way ANOVAs with aggression group and delta prolactin group as between-group factors. These analyses revealed significant main effects for aggression group in the percentage of parents and aunts and uncles with aggressive behaviors (parents: $F= 20.42$, $df = 1,87$, $p < 0.0001$; aunts and uncles: $F = 6.03$, $df = 1,87$, $p = 0.02$). There was also a trend towards an aggression group effect on the percentage of aggressive grandparents ($F = 2.94$, $df = 1, 87$, $p = 0.09$). In addition, the aggression group-by-delta prolactin group interaction was significant for the percentage of aggressive parents ($F = 5.20$, $df = 1,87$, p

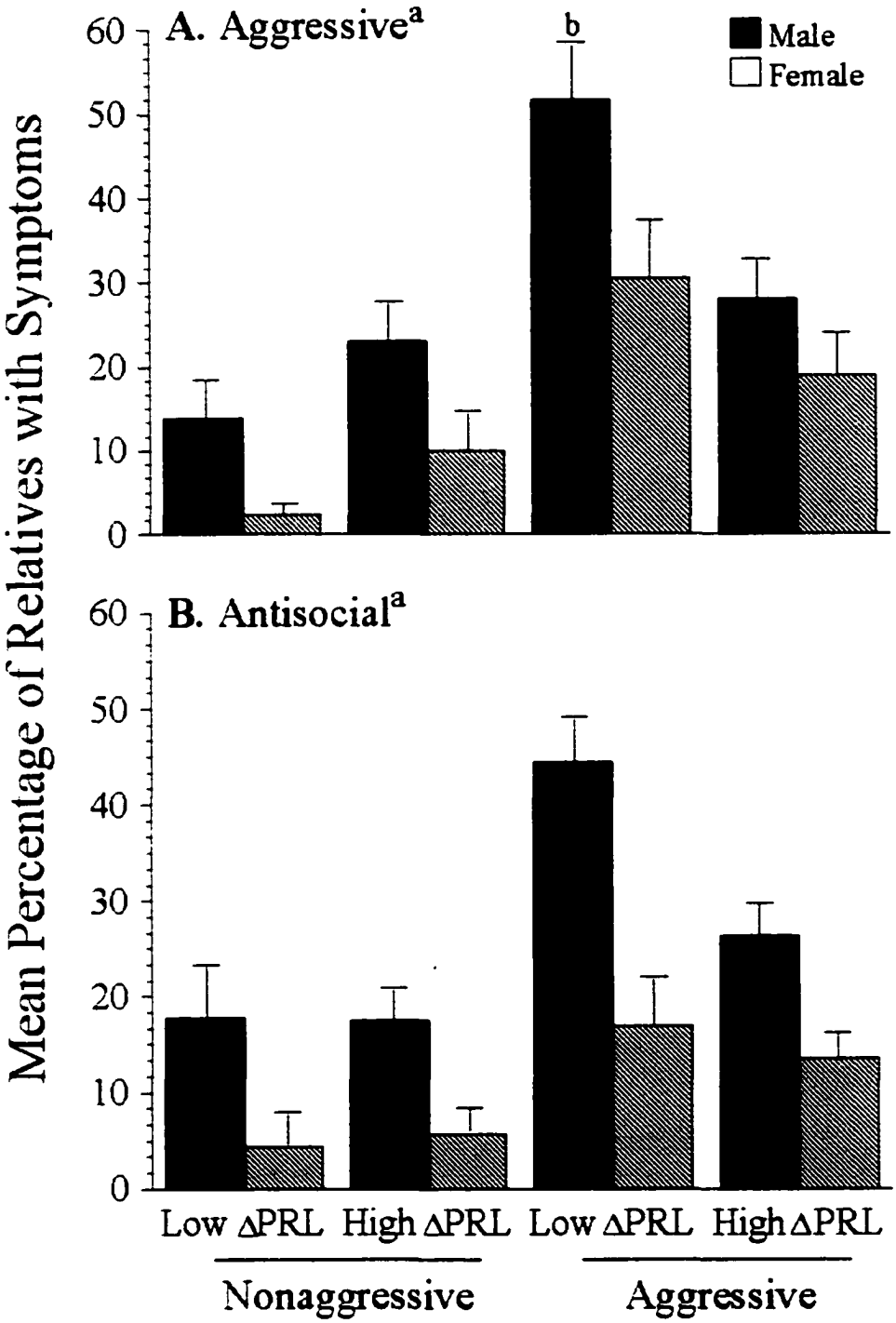

Figure 7. Gender differences in the mean percentage of relatives with A) aggressive and B) antisocial behavior as a function of child aggression and peak delta prolactin response to fenfluramine (ΔPRL).

[a] Main effects for gender and aggression group, $p < 0.01$.

[b] Significantly greater than other three groups, $p < 0.01$.

Table 12. Odds ratios and 95% confidence intervals (CI) for aggressive and antisocial histories in first and second degree relatives as a function of child aggression and peak delta prolactin response to fenfluramine (ΔPRL)

	Relatives w/					
	Aggressive History			Antisocial History		
Contrasts	Odds Ratios	95% CI	*p*	Odds Ratios	95% CI	*p*
Aggressive vs. Nonaggressive	3.11	2.13 - 4.54	<0.001	2.58	1.76 - 3.81	<0.001
Low ΔPRL vs. High ΔPRL	0.84	0.61 - 1.15	0.27	1.41	1.00 - 1.99	0.048
Aggressive/Low ΔPRL vs. Aggressive/High ΔPRL	1.73	1.18 - 2.53	0.005	1.85	1.23 - 2.78	0.003
Aggressive/Low ΔPRL vs. Nonaggressive	4.10	2.69 - 6.24	<0.001	3.52	2.29 - 5.41	<0.001
Aggressive/High ΔPRL vs. Nonaggressive	2.37	1.54 - 3.64	<0.001	1.90	1.22 - 2.98	0.004
Aggressive/Low ΔPRL vs. Nonaggress/Low ΔPRL	5.20	3.00 - 9.02	<0.001	3.61	2.13 - 6.12	<0.001

= 0.03). The aggression group-by-delta prolactin group interactions for the percentage of aggressive aunts and uncles and grandparents were in the same direction, but did not reach significance (both $p > 0.10$). The ANOVAs assessing the percentage of relatives with antisocial behaviors yielded a significant main effect for aggression group on the percentage of antisocial parents ($F = 7.25$, $df = 1,87$, $p = 0.009$), but no other significant main or interaction effects (all $p > 0.10$).

Table 12 presents the odds ratios of having a relative with a positive history for aggressive and antisocial behaviors as a function of aggression group and delta prolactin group. The odds ratios were significantly greater for aggressive boys than for nonaggressive boys. Further, aggressive boys with low delta prolactin: a) were nearly twice as likely to have an aggressive relative than aggressive boys with high delta prolactin; b) had a three- to fourfold increase in the odds of having an aggressive and/or antisocial relative compared to nonaggressive boys, irrespective of delta prolactin; and c) were three to five times as likely to have an aggressive and/or antisocial relative than nonaggressive boys with low delta prolactin specifically. However, low delta prolactin by itself did not significantly increase the odds of having an aggressive or antisocial relative

DISCUSSION

These data indicate that although there is no direct relationship between reduced 5-HT function and aggression in prepubertal children, a subgroup of these children do in fact have relatively low central 5-HT activity. Further, relatively low central 5-HT

function is associated with aggressive behavior in first- and second-degree family members, suggesting that it may represent a familial risk factor for aggression. The findings were uniform for male and female relatives. In contrast, antisocial characteristics were more frequent among male and female relatives of aggressive boys, but did not seem to be associated with reduced central 5-HT activity. These results were specific to aggressive and antisocial characteristics in the relatives; the frequency of ADHD symptoms, cognitive impairment/learning problems and internalizing symptoms did not differ as a function of child aggression and/or prolactin response to fenfluramine.

These data support the hypothesis that familial aggressive behavior is associated with central 5-HT activity in aggressive boys. The high prevalence of aggressive characteristics that is often reported in the families of aggressive children was primarily accounted for by those children with diminished central 5-HT activity. Specifically, the incidence of aggressive behaviors among relatives of aggressive boys with low central 5-HT activity was four times greater than the incidence in relatives of nonaggressive children and nearly double the rate in relatives of aggressive children with high central 5-HT. Further, this elevated incidence of aggressive behavior was seen among the parents, and to a lesser extent, the aunts and uncles of boys with low central 5-HT activity. A similar pattern of results was noted when antisocial characteristics among the relatives were examined, but the magnitudes of the findings were smaller and, for the most part, not significant. These findings suggest that aggressive behavior may be more biologically determined than antisocial behavior, which may be influenced more by the familial environment.

The current findings are partially consistent with those from a study in personality

disordered adults that linked low central 5-HT activity to high rates of impulsive aggressive traits among first-degree relatives (Coccaro et al., 1994). However, that study found that a blunted prolactin response to fenfluramine was related to increased prevalence of familial impulsive aggression irrespective of the proband's behavior. In contrast, a blunted prolactin response was relatively frequent among nonaggressive boys in the current sample and was by itself not associated with familial aggression. Rather, a blunted prolactin response was associated with an increased frequency of aggressive characteristics only among the relatives of aggressive boys. In fact, among boys with low central 5-HT activity, the presence of childhood aggression increased the odds of having an aggressive relative by five-fold. Thus, reduced central 5-HT activity alone is unlikely to be sufficient to account for the presence of aggression in children.

These data point to the possible existence of distinct familial and nonfamilial subgroups of aggressive boys that differ with regard to central 5-HT activity. Specifically, familial aggression was associated with a subgroup of aggressive boys who presented with relatively lower central 5-HT activity. In contrast, aggressive boys with higher central 5-HT were not characterized by an abundance of aggressive relatives. The presence of distinct subgroups of aggressive boys could potentially explain the discrepancies between and within the studies of central 5-HT activity and childhood aggression (Castellanos et al., 1994; Halperin et al., 1994, 1997a; Kruesi et al., 1990, 1992; Pine et al., 1997; Stoff et al., 1992), as well as help reconcile these inconsistent findings with the consistent evidence of diminished central 5-HT function in aggressive adults (reviewed in Zubieta & Alessi, 1993).

The findings of the current study must be considered in the context of the

increased prevalence of mood disorders among the aggressive boys with reduced central 5-HT activity. Mood disorders have been consistently associated with diminished central 5-HT activity in both children (Birmaher et al., 1997; Ryan et al., 1992) and adults (Siever et al., 1984), and might potentially contribute to the reduced central 5-HT activity among these boys. However, the relatively specific relationship between reduced central 5-HT activity and familial aggression combined with the lack of an association between reduced central 5-HT and internalizing symptoms among family members makes this notion seem rather unlikely. Similar to the current findings, reduced central 5-HT activity in personality disordered adults, who also had a high prevalence of mood disorders, was associated with impulsive aggression and not mood symptoms among relatives (Coccaro et al., 1994).

GENERAL DISCUSSION

These data constitute the largest collection of evidence to date on the role of central 5-HT responsivity in the manifestation of aggressive behavior in children. The lack of a clear and direct relationship between central 5-HT activity and aggressive behavior among these boys is consistent with several other studies that also failed to find such an association in children (Clarke et al., 1999; Constantino & Murphy, 1996; Stoff et al., 1992). Even those studies that have noted a relationship between central 5-HT and aggression in children are inconsistent in the direction of the findings, with some reporting a positive relationship (Castellanos et al., 1994; Pine et al., 1997) and others reporting an inverse association (Kruesi et al., 1990, 1992). The variability and overlap among aggressive and nonaggressive subjects in these studies has even led some to question the utility of measuring central 5-HT activity in relation to aggression in children. Notwithstanding these criticisms, the present analyses indicate that although there is no direct relationship between reduced 5-HT function and aggression in prepubertal boys, relatively lower central 5-HT function in aggressive boys is associated with familial risk factors for the progression of aggressive behavior. In contrast, neither seasonally-related variance nor developmental and diagnostic factors seem to influence the impact that central 5-HT has on aggression in children. The findings with regard to each of these factors are discussed in more detail below.

Seasonally-Related Variance. The present results do not confirm the hypothesis that aggressive and nonaggressive boys with ADHD exhibit distinct seasonal fluctuations in central 5-HT activity. There was no evidence of seasonal variations in central 5-HT

responsivity in the entire sample of boys with ADHD or in separate aggressive and nonaggressive subgroups of these boys. The absence of group-specific seasonal differences in central 5-HT activity suggests that seasonally-related variance does not account for the considerable overlap among the aggressive and nonaggressive boys in the current sample. While these analyses do not support the hypothesis that seasonally-related variance accounts for the discrepancy among the child studies of central 5-HT, these data can not rule out the existence of seasonal variations in central 5-HT activity in other studies of child aggression.

The lack of seasonality in central 5-HT responsivity in children with ADHD stands in contrast to reports of seasonal fluctuations in 5-HT in suicidal adolescents (Pine et al., 1995) and depressed and suicidal adults (Arora et al., 1984; Cappiello et al., 1996). This discrepancy may be related to differences in the symptom course between the samples studied. Seasonal trends in depressive symptomatology (Maes et al., 1993a) and the prevalence of suicide (Maes et al., 1993b, 1995) have been consistently reported and may partially account for the seasonal effects in 5-HT function found in these patient groups (Maes et al., 1995, 1996). In contrast, there are no reports in the literature indicating seasonal differences in the symptomatology of ADHD, and assuming markers of 5-HT activity follow pathology and not some other factor, no seasonal differences in markers of 5-HT would be expected. Unfortunately, the lack of a control group in the current study precluded determining whether the lack of seasonal variation in central 5-HT function is specific to ADHD or is also seen in psychiatrically-healthy boys.

Diagnostic Factors: Presence of ADHD. The current set of results also fail to support the hypothesis that the relationship between central 5-HT function and

aggression varies as a function of the presence or absence of ADHD. Central 5-HT function did not differ between groups of nonaggressive boys with ADHD, aggressive boys with ADHD, and aggressive boys without ADHD and was unrelated to severity of ADHD. More importantly, statistically removing the potential impact of ADHD did not influence the lack of a relationship between central 5-HT responsivity and aggression. These analyses indicate that it is highly unlikely that diagnostic differences across samples, specifically in the proportion of subjects with ADHD, account for the variable results of studies assessing central 5-HT activity in boys.

These results are not particularly surprising given that two studies had already shed some light on this issue. These studies separately reported positive associations between central 5-HT activity and aggression in children measuring CSF 5-HIAA (Castellanos et al., 1994) and the prolactin response to fenfluramine (Pine et al., 1997). The sample in the former study was specifically recruited for the presence of ADHD, raising the possibility that ADHD does account for the unexpected direction of the finding (Castellanos et al., 1994). To help understand their findings, these investigators systematically compared their sample to a previous sample from the same laboratory (Kruesi et al., 1990), in which an inverse relationship was found between CSF 5-HIAA and aggression. This comparison indicated that the sample in the later study was younger, less aggressive, and more hyperactive. Any of these variables may have accounted for the difference in the direction of the findings. However, the majority of the subjects in the earlier study also had ADHD, making this factor less likely to account for the difference in findings. The sample in the second study that reported a positive association between central 5-HT activity and aggression consisted of siblings of

adjudicated adolescents, many of whom presented with no diagnosis (Pine et al., 1997). This sample was relatively young, relatively nonaggressive, and primarily not ADHD. Thus, it was unlikely that the presence of ADHD accounted for the positive association in this sample. Moreover, statistical analyses revealed no association between ADHD and central 5-HT responsivity in this relatively unimpaired sample (Pine et al., 1997).

Studies using peripheral indices of 5-HT also suggest that the presence of ADHD does not impact on the relationship between 5-HT and aggression in children. Only one (Stoff et al., 1987) of six studies that examined peripheral 5-HT in boys with ADHD (Cook et al., 1995; Pornoppal et al., 1999; Spivak et al., 1999; Stoff et al., 1987, 1989, 1991) reported an association between 5-HT and aggression, and this finding was not replicated when the sample was expanded (Stoff et al., 1991).

Developmental Factors. The current results do not support the hypothesis that the relationship between central 5-HT and aggression in boys with ADHD changes as a function of age. There was no evidence of age-related variations in the association between central 5-HT activity and aggression in boys with DBD. In addition, central 5-HT activity did not differ between older and younger boys and was unrelated to age. These findings indicate that inconsistencies across the child studies of central 5-HT and aggression are not likely to be accounted for by developmental differences across samples. Yet, the narrow age range of the current sample and the use of a cross-sectional design makes this conclusion tentative.

The present results are incompatible with previous analyses that found age-related changes in the association between central 5-HT responsivity and aggression among the boys in Sample 1 (Halperin et al., 1997a). Central 5-HT activity was enhanced in young

aggressive boys relative to young non-aggressive boys with ADHD, but did not differ among slightly older boys. Moreover, central 5-HT activity decreased over a two-year period in a subgroup of these boys (Pick et al., 1999). This subgroup was too small (N = 10) to elucidate meaningful group differences in developmental trajectory, but boys who were aggressive at both time points tended to have the greatest drop in central 5-HT activity. This finding is in contrast to our cross-sectional results, where the age-related changes occurred in the nonaggressive group.

The absence of age effects in the current study is also inconsistent with numerous studies that have demonstrated age-related changes in 5-HT activity in humans. Non-linear developmental changes in both peripheral and central markers of 5-HT activity have been described in humans, such that enhanced activity in early childhood is followed by a decline through puberty (Biegon & Greuner, 1992; Hedner et al., 1986). Inverse correlations between age and CSF 5-HIAA levels have also been observed (Hyland et al., 1994; Kruesi et al., 1990). However, only one study has addressed the issue of differential changes in aggressive and nonaggressive boys. Platelet 5-HT_2 receptor binding was reduced in 13 to 14 year-old incarcerated adolescents compared to controls, but binding decreased with age in the controls such that the difference was negligible between the 17 to18 year-old groups of delinquents and controls (Blumensohn et al., 1995).

In summary, these data suggest that despite the negative results of the current analyses, age-related changes may be important for reconciling differences across various studies assessing the relationship between 5-HT and aggression in children. Furthermore, developmental changes in 5-HT function could provide a link between the

child and adult data. In adults, most data suggest decreased central 5-HT function in aggressive relative to non-aggressive subjects, whereas in children findings have been inconsistent.

Familial Factors: Familial Aggression. The current results support the hypothesis that familial aggressive behavior varies as a function of central 5-HT activity in aggressive boys. The high prevalence of aggressive characteristics that is often reported in the families of aggressive children was almost entirely accounted for by those boys with diminished central 5-HT activity. Specifically, the incidence of aggressive behaviors among relatives of aggressive boys with low central 5-HT activity was four times greater than the incidence in relatives of nonaggressive boys and nearly double the rate in relatives of aggressive boys with high central 5-HT. However, low central 5-HT activity by itself was not associated with familial aggression. These findings were evident in first-degree relatives and were uniform across male and female family members. A similar pattern of results was noted when antisocial characteristics among the relatives were examined, but the magnitudes of the findings were smaller and did not reach significance. Thus, these data can not confirm the hypothesis that familial antisocial behavior varies as a function of central 5-HT activity. Yet, the data can also not rule out the hypothesis either.

The current analyses extend the previous finding of an association between diminished central 5-HT activity and parental history of aggression in a subgroup of the aggressive boys in this sample (Halperin et al., 1997b). Aggressive children with at least one aggressive parent had a blunted prolactin response to fenfluramine compared to nonaggressive children, whereas aggressive children without a positive history of

parental aggression had an enhanced response relative to nonaggressive boys. Further, aggressive children of aggressive parents were more aggressive than their counterparts without aggressive parents. The confluence of more severe aggressive behavior and a parental history of aggression in a subgroup of aggressive boys suggests that these boys are at greatest risk for progression to more serious violence in adolescence and adulthood. The fact that this group also had low 5-HT function raises the possibility that low central 5-HT activity in aggressive children is associated with poor outcome.

Taken together, these analyses seem to indicate the existence in the current sample of distinct familial and nonfamilial subgroups of aggressive boys that differ with regard to central 5-HT activity. Specifically, familial aggression was associated with a subgroup of aggressive boys who presented with reduced central 5-HT activity. The presence of neurochemically distinct subgroups of aggressive boys could account for the wide variability within and between the child studies of central 5-HT activity and aggression (reviewed in Mitsis et al., 2000), as well as help reconcile these findings with the consistent evidence of diminished central 5-HT function in aggressive adults (reviewed in Zubieta & Alessi, 1993). Since only about half of aggressive boys progress to serious violence as adults, while the remainder desist in their aggression, many young boys who are classified as aggressive will likely desist in their aggression later in life and may not have the same neurobiology as those boys whose aggression will escalate. The relationship between central 5-HT function and aggression may be obscured enough in childhood by these phenocopies so that it is not readily evident, but may become apparent as the phenocopies desist in their aggression during adolescence. This line of thinking raises the possibility that diminished central 5-HT activity might be an

important risk factor for the persistence of aggression into adolescence and adulthood (Halperin et al., 1997b).

LOW CENTRAL 5-HT ACTIVITY: RISK FACTOR FOR PERSISTENCE IN AGGRESSIVE BOYS?

Early physical aggression is a reliable predictor of subsequent violent behavior in adulthood, and most violent adults have a history of childhood aggression (Farrington, 1991), but only a small proportion of young boys who present with aggression escalate to serious violence as adults (Loeber et al., 1993). Familial aggression is an important risk factor for the escalation of childhood aggression into adult violence (Cadoret et al., 1995; Frick et al., 1992). The fact that familial aggression was associated in the current study with a subgroup of aggressive boys who presented with reduced central 5-HT activity raises the possibility that diminished central 5-HT activity (which is characteristic of aggressive adults) constitutes a risk factor for persistence in aggressive boys. A corollary hypothesis is that aggressive boys with higher central 5-HT function are more likely to desist in their aggressive behavior later in life.

Previous studies involving a subgroup of the current sample provide further support for the hypothesis that aggressive boys with diminished central 5-HT activity are at increased risk for poor outcome. These studies linked low central 5-HT with other risk factors for persistence in aggressive boys, namely cross-situationality of aggression (McKay et al., 1996) and emotional lability (Newcorn et al., 1996). Consistent with data indicating that diversity of settings in which aggression occurs is a risk factor for greater

violence (Loeber et al., 1993), boys who were described as pervasively aggressive (at home *and* school) had a blunted prolactin response to fenfluramine compared to boys who were situationally aggressive (at home *or* school) (McKay et al., 1996). Other analyses in the same sample of aggressive boys revealed that prolactin response was inversely correlated with ratings of emotional lability (i.e., impulsive, irritable, mood swings, hot temper) and was lower in boys rated as emotionally labile compared to boys who were not emotionally labile (Newcorn et al., 1996). These findings provide continuity with studies in personality disordered adults that consistently report an inverse association between central 5-HT activity and measures of impulsivity, irritability, and affective aggression (Coccaro et al., 1989, 1992, 1995, 1996b, 1997c, 1997d).

According to this line of thinking, indices of central 5-HT function in prepubertal boys may not be highly correlated with aggressive behavior. Instead, diminished central 5-HT activity may be limited to only a subgroup of aggressive children, presumably those most likely to persist in their aggression. Conversely, aggressive children with higher central 5-HT activity, which is not associated with risk factors for persistence in children or with aggression in adults, may be more likely to have a remission of their aggressive behavior as they mature. Seen in this light, examining central 5-HT activity may have prognostic value in boys, as exemplified by Kruesi et al. (1990, 1992), who found that not only did low CSF 5-HIAA predict aggression at two-year follow-up in disruptive boys, but that the magnitude of this association increased over the follow-up period. Similarly, newborn CSF 5-HIAA was not related to infant behavior, but was inversely related to familial APD and the associated risks for the development of antisocial behavior (Clarke et al., 1999). Thus, the frequently reported variability in

central 5-HT activity in aggressive boys may have predictive value with regard to long-term outcome and may be related to the fact that only about half of aggressive children progress to violent behavior as adults (Loeber et al., 1993). However, these hypotheses can only be adequately tested with a prospective study that follows aggressive children into adulthood.

LOW CENTRAL 5-HT ACTIVITY AND FAMILIAL TRANSMISSION OF AGGRESSIVE BEHAVIOR

The idea that aggressive tendencies are transmitted through families has received considerable scientific support over the last three decades. Family studies have consistently reported a two- to threefold increase in the incidence of aggressive behavior among the first- and second-degree relatives of aggressive boys compared to clinic controls (Hamdan-Allen et al., 1989; Lahey et al., 1988; Stewart & Leone, 1978). The results of the present analyses suggest that this elevated incidence of familial aggression is likely to be predominately accounted for by those aggressive children who present with diminished 5-HT function. Aggressive boys with low central 5-HT activity in the current study had nearly double the rate of aggression in relatives compared to aggressive boys with high central 5-HT and accounted for the majority of aggressive relatives in the sample. These data suggest that aggressive boys with low central 5-HT activity represent an endophenotype, and raise the possibility that the familial transmission of aggressive behavior is mediated in part by diminished central 5-HT activity.

Sophisticated adoption and twin studies have provided robust evidence that

aggressive behavior is heritable and partially determined by genetic influences (Cadoret et al., 1995; Coccaro et al., 1997a). Genetic factors seem to exert a moderate to strong influence on the expression of aggression, accounting for 28% to 47% of the variance (Coccaro et al., 1997a). Recent research has even identified several 5-HT system genes as potential candidate genes for both impulsive aggression (Mannuck et al., 1999, 2000) and the prolactin response to fenfluramine (Mannuck et al., 2000; Reist et al., 2001). However, other research suggests that both aggressive behavior and diminished 5-HT activity could just as plausibly result from environmental factors associated with having aggressive parents, as exemplified by the association between 5-HT, aggression, and adverse rearing reported by Pine et al. (1996, 1997). Unfortunately, the data from the present study cannot definitively determine the extent to which genetic or environmental influences account for the high rate of aggressive relatives among aggressive children with diminished central 5-HT. Nonetheless, the fact that the aggressive boys with low central 5-HT had a significantly elevated incidence of aggressive characteristics in parents, but not in aunts, uncles, and grandparents suggests an interaction of genetics and environmental influences. However, the fact that as many as 10 of the 25 (40%) aggressive boys with low central 5-HT activity did not live with an aggressive adult tentatively points to genetic factors. Finally, the relatively low prevalence of aggression among the relatives of nonaggressive children with low central 5-HT is noteworthy and suggests that diminished central 5-HT activity may not be associated with physical aggression per se, but rather with some underlying, related behavioral characteristic, such as poor impulse control.

STUDY LIMITATIONS

The findings of the present study and any interpretations of these data must be considered in the context of the methodological limitations of the studies. Some of these limitations have already been discussed previously, namely the modeling of seasonal rhythms using single samples and the possible non-representativeness of small subject groups. However, methodological limitations also exist concerning the fenfluramine challenge procedure, the family history interview, and the general study design. Each of these limitations will be discussed briefly in turn.

Notwithstanding the large body of research supporting the scientific basis of the fenfluramine challenge procedure, there are several methodological limitations of the specific procedures used in this study. First, ethical and practical considerations prevented the inclusion of psychiatrically healthy children as a control group in this study. The lack of a control group precludes drawing any conclusions from this study regarding whether high or low central 5-HT activity as defined in this study represents deviant brain function. Second, placebo challenges were not performed as part of this study. However, studies in both adolescents and young adults have demonstrated that the robust rise in plasma prolactin following acute fenfluramine administration is due to the effects of the medication and not to non-specific hormonal changes associated with the procedure itself (McBride et al., 1989; Stoff et al., 1992). Placebo challenge only minimally altered plasma prolactin levels in these studies. Third, the racemic isomer of fenfluramine used in the current study to assess central 5-HT activity is also known to impact on dopamine transmission, although these effects have only been reported with

substantially higher doses of *d,l*-fenfluramine than used presently (Meyerdorff et al., 1986). In fact, the prolactin responses to the dose of *d,l*-fenfluramine used in the current study are highly correlated with prolactin response to comparable doses of the more 5-HT-selective *d*-fenfluramine in adults (Coccaro et al., 1997d). Finally, the prolactin response to fenfluramine is considered to reflect overall 5-HT function specifically in the hypothalamic-pituitary axis (reviewed in Cowen et al., 1990). Thus, the results of the current study can not determine the extent to which aggression in children may or may not be related to 5-HT activity in other brain regions (e.g., orbitofrontal cortex).

The method used to determine family history in the current study also has a number of methodological limitations. First, the procedure relied primarily on the report of a single informant, which has been found to result in underreporting of symptomatology (Andreasen et al. 1977). However, reliance on data from a single informant has been found to be more sensitive (i.e., less underreporting) when diagnosing overt acts such as aggressive behavior compared to diagnosing psychiatric disorders defined by internal states (Thompson et al. 1982). Further, sensitivity is increased only slightly when using multiple informants, as errors made by different informants tend to be correlated. Second, the actual interview used to determine family history is retrospective in nature and may have been subject to bias, forgetting, and limited knowledge on the part of the respondent (Andreasen et al. 1977). Nevertheless, these sources of error variance would be expected to decrease, not increase, the likelihood of finding significant results. While being aggressive and having an aggressive child are known to increase the likelihood of reporting an aggressive history in other family members (Chapman et al., 1994), this bias could not account for the increased rate of

aggression only in the relatives of aggressive children with diminished 5-HT activity, because both the interviewer and informant were unaware of the biological data.

The general design of the three studies in the current analyses also present some limitations on the conclusions that can be drawn from the findings. These analyses are premised on certain assumptions about the development of both central 5-HT activity and aggressive behavior over time in prepubertal boys. Yet, the cross-sectional design of the current studies does not permit direct testing of these hypotheses, which can only be adequately tested with a longitudinal study that follows aggressive boys both biologically and behaviorally into adulthood. The results of these analyses may also have been limited somewhat by the reduced statistical power generated by splitting the sample into small subgroups. This reduction in power may be the reason that the findings regarding familial antisocial behavior did not quite reach significance in the third study.

Notwithstanding these methodological limitations, these results add to our knowledge regarding the biological factors that may be involved in the development of aggressive and antisocial behavior in prepubertal boys.

FUTURE DIRECTIONS

The fact that fenfluramine is no longer available as a probe of central 5-HT activity effectively precludes replication of these results and complicates future studies of the role of central 5-HT function in childhood aggression. However, the hypothesis that low central 5-HT activity constitutes a risk factor for persistence in aggressive boys can be tested with a prospective study that follows these aggressive children behaviorally into

adulthood. Moreover, the hypothesis that a particular pattern of central 5-HT activity is associated with familial aggression can be pursued using emerging neuroimaging (e.g., PET) techniques as well as genetic approaches.

CONCLUSIONS

Considerable research has established low brain 5-HT activity as a biological risk factor for aggressive, impulsive, and antisocial behavior in animals and human adults. Diminished central 5-HT activity also plays a role in the manifestation of childhood aggression, but the few studies conducted in children have yielded inconsistent results. The fact that aggressive boys are neither uniformly "high" nor "low" with regard to central 5-HT suggests that there is no clear and direct relationship between diminished central 5-HT activity and aggression in prepubertal boys. Yet, the present analyses indicate that among aggressive boys, relatively lower central 5-HT function is associated with familial risk factors for the progression of aggressive behavior. In contrast, aggressive boys with relatively higher central 5-HT did not have risk factors for persistence. Since only about half of aggressive boys progress to adult violence, and aggressive men tend to have low central 5-HT activity, these data provide compelling support for the hypothesis that low 5-HT function may be a marker or risk factor for such progression in aggressive prepubertal boys. These results also raise the possibility that the familial transmission of aggressive behavior may be mediated in part by diminished central 5-HT activity. However, these hypotheses can only be adequately tested with a

prospective study that follows aggressive children both biologically and behaviorally into adulthood.

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