OTHER CONSIDERATIONS

OVERVIEW

This chapter briefly presents evidence related to several topics explored in lesser detail. These topics include fertility, hormonal and stress effects, sleep, mood, dermatologic effects, birth defects, and violent death.

While the cholinergic system is involved in reproduction, and thus alterations in fertility might be theoretically possible, there is presently no evidence of reduced fertility in PGW veterans.

PB is known to affect certain hormones, including growth hormone. No evidence has been identified to suggest that growth hormone abnormalities are associated with illnesses in PGW veterans, though there is no evidence to exclude this possibility.

PB affects the cholinergic system, which in turn affects sleep, and many PGW veterans complain of sleep abnormalities. Increased incidence of sleep apnea may occur in ill PGW veterans, and studies suggest that nicotinic cholinergic stimulation may ameliorate symptoms of sleep apnea. Nonetheless no direct evidence supports or excludes the possibility that PB use alone or in conjunction with exposures to other AChE inhibitors is associated with current sleep complaints among veterans. Sleep abnormalities merit further evaluation because of the possible link to the one identified source of excess mortality in deployed PGW veterans, namely death by unintentional injury.

Many ill veterans report rashes, hair loss, or other symptoms related to the dermatologic system. One prior report characterizes a woman who repeatedly experienced hair loss on institution of PB for myasthenia. Literature suggests some mechanisms by which skin effects could occur. Although it is not possible to attribute skin symptoms or hair loss in ill veterans to use of PB, neither is it possible to exclude PB as a contributor to reported skin symptoms and hair loss.
No literature suggests severe birth defects with PB use in pregnant myasthenics. PB use in myasthenia may be linked to the development of neonatal myasthenia; however, this effect occurs with high-dose PB delivered for long periods. Moreover, epidemiologic evidence does not suggest increased rate of severe birth defects in children of PGW veterans. Risk of birth defects was increased (borderline significance: RR 1.12, 95 percent CI 1.00–1.25) in children of deployed women, although the increase was no longer significant after adjustment for race/ethnicity, marital status, and branch of service (Cowan, De Fraites, et al., 1997). PB has been shown to demonstrate some teratogenic of developmental effects in rats and chickens. Gross structural effects were seen in chickens exposed to high doses of PB. More subtle behavioral effects and regional brain function changes were seen in rats exposed to low doses of PB in their neonatal period, doses more comparable to exposures occurring in PGW veterans.

PGW veterans have experienced increased death by unintentional injury. While many factors could contribute to this increase, subjective complaints of difficulties with sleep and concentration by ill veterans—complaints that may or may not relate to abnormal ACh function and prior PB use—merit scrutiny as contributing factors.

FERTILITY

Data identifying the presence of ACh and AChE in sperm, ovaries, and other reproductive tissues suggest a role for the cholinergic system in reproduction, leading to the question whether alterations of cholinergic activity could produce changes in fertility. Ongoing fertility problems would likely (but not necessarily) require the presence of long-standing changes in cholinergic function following exposure to PB (and other AChE-inhibiting coexposures, such as a nerve agent or pesticides). It is unknown whether such long-standing changes occur.

A role for cholinergic function in reproduction is suggested by the presence of cholinesterases in ovarian follicles, cholinergic villi, and human oocytes, and cholinergic signaling has been implicated in chorionic villi, which express the BChE gene, and in sperm motility, as suggested by presence of ACh, AChE, and choline acetyltransferase, as well as BChE in mammalian sperm. One µM of an agent that inhibits ACh production depressed sperm motility by 95 percent; while ACh itself may either augment or depress sperm motility (Schwarz, Glick, et al., 1995).

An effect of PB on fertility has not been demonstrated. Following return from the Gulf, PGW veterans experienced a higher birth rate then nondeployed controls. While it is conceivable that this increased birth rate was less than the
increase in procreation behavior would warrant, there is no evidence to allow assessment of this possibility. If concerns regarding fertility remain, birth rates farther out from the time of deployment should be evaluated.

**HORMONE AND STRESS EFFECTS**

Differences in the influence of PB on growth hormone or other pituitary-adrenal axis hormones could themselves be postulated to play a role in individual differences leading to illnesses in some PGW veterans and not in others. PB is used in the evaluation of growth hormone status and acts to cause a surge of growth hormone presumably by inhibiting release of somatostatin, a growth hormone suppressant, from a region of the brain termed the hypothalamus (Wehrenberg, Wiviot, et al., 1992). Marked differences in growth hormone response to PB have been documented; these may interact with other factors that influence hypothalamic regulation of growth hormone secretion, as noted above. Moreover, PB interacts with other factors that regulate functional growth hormone release. For instance, PB administration significantly augments the exercise-induced increase in growth hormone release (Cappa, Grossi, et al., 1993) and the delayed growth hormone response to glucose administration (Valcavi, Zini, et al., 1992); it also partially reverses the inhibition of growth hormone response to the growth hormone–releasing hormone found in corticosteroids (Trainer, Kirk, et al., 1991).

In turn, differences in growth hormone may influence other systems in the body. For instance, growth hormone is involved in regulation of blood glucose, muscle mass response to exercise, and has been shown to enhance cardiac function (Valcavi, Gaddi, et al., 1995).

More generally, acetylcholinergic stimulation (e.g., with physostigmine) appears capable of producing a stress response in which cortisol, prolactin, and growth hormone are all elevated (Davis and Davis, 1979). (Corticosteroids, such as cortisol, are considered the quintessential stress hormones, although in acute stress, catecholamines are released as well. AChE inhibitors also promote release of catecholamines—see below.) In vitro studies suggest stimulation of corticotropin-releasing factor from the hypothalamus in rats (Bradbury, Burden, et al., 1974). The effect is believed to be largely nicotinic, since nicotinic blockade reduces the effect while a muscarinic agent (bethanecol) had no effect (Hillhouse, Burden, et al., 1975). (Although a number of studies suggest that the stress-induced secretion of cortisol has a muscarinic acetylcholinergic basis, there are reasons to doubt this. For instance, infusion of the muscarinic antagonist atropine into the fluid-filled ventricles of the brain (or implanting of atropine pellets into the hypothalamus of the brain) inhibits the stress response. However, this does not occur when doses of atropine are used that
are specific for blockade of muscarinic receptors (0.4 mg/kg) (Davis and Davis, 1979). More data on stress and the cholinergic system are provided in a footnote.1

PB may influence other hormones as well, perhaps including calcitonin and TRH (the hormone from the hypothalamus that incites release of thyroid-stimulating hormone from the pituitary). PB (120 mg orally, or four Gulf War doses) has been shown to increase plasma levels of calcitonin gene-related peptide in normal humans (p < .01) (Trasforini, Margutti, et al., 1994). If cholinergic “downregulation” (see Chapter Three, “Characteristics of PB”) occurs following PB withdrawal, the effect on calcitonin may be viewed with interest in PGW veterans who report pain, in light of increasing evidence for, and increasing specialist use of, calcitonin as a pain-reducing agent. (Of more direct relevance is evidence that nicotinic ACh function is perhaps more directly involved in pain control—discussed in greater detail in the section on pain, below.) PB suppresses hypothalamic somatostatin; and somatostatin suppresses thyroid-stimulating hormone from the pituitary. Tests indicate that PB (at a dose of 180 mg; six Gulf War doses, or two “daily” doses) can augment the thyroid-stimulating hormone response to TRH in a normal man (Yang, Woo, et al., 1995). Others find effects on the thyroid-stimulating hormone response to TRH only in patients with Cushing’s disease (Giustina, Bossoni, et al., 1992). No data have evaluated whether subtle long-term effects may characterize thyroid hormone function or response following subchronic use of PB.

1 Administering the ACh-mimicking drug “arecoline” (at 4 mg/kg) to rats increased elevation of serum corticosterone particularly in rats bred to be sensitive to ACh (Overstreet, Janowsky, et al., 1986). (It also produced greater suppression of behavioral activity in ACh-sensitive rats (Overstreet, Janowsky, et al., 1986), which show more immobility in the face of stress to begin with (Overstreet, Janowsky, et al., 1986).)

Of note: rats chronically treated with and subsequently withdrawn from the muscarinic blocking drug scopolamine (2 mg/kg once daily) or the antidepressant drug amitriptyline (10 mg/kg once daily), which has muscarinic actions, were also significantly more immobile (Overstreet, Janowsky, et al., 1986; Overstreet, Russell, et al., 1988), consistent with the idea that depressing the cholinergic system with drugs may render it more sensitive on withdrawal.

Rats bred for sensitivity to cholinergic agonists, the Flinders Sensitive Line (FSL) rats, exhibited on autopsy lower concentrations of the Corticotropin Releasing Factor receptors in the median emi-
nence, locus ceruleus, and prefrontal cortex but not in 13 other brain regions (Owens, Overstreet, et al., 1991). (The Corticotropin Releasing Factor is released by the hypothalamus and stimulates the pituitary to release the adrenocorticotropic hormone, which in turn stimulates the adrenal glands to release corticosteroids.) They had significantly lower plasma adrenocorticotropic hormone concentration, but no difference in corticosterone in comparison to Flinders Resistant Line rats (Owens, Overstreet, et al., 1991). The density of Corticotropin Releasing Factor receptors in the anterior pituitary was elevated. This was taken to suggest that cholinergically “supersensitive” Flinders Sensitive Line rats may have diminished hypothalamic-pituitary-adrenal activity (Owens, Overstreet, et al., 1991).
A variety of hormonal effects have been noted following exposure to other AChE inhibitors, specifically to the nerve agent soman, including increases in serum corticosterone, thyroxine, and triiodothyronine concentrations; reduction in adrenocorticotropic hormone levels; and reduction in testosterone (Clement, 1985). Whether long-standing hormonal effects occur with AChE inhibitors has not been evaluated. Specifically, studies have not been identified that evaluate chronic effects on hormones from PB alone or in combination with other AChE inhibitors.

One of the more widely touted hypotheses with regard to illnesses in PGW veterans has been the stress hypothesis. Some postulate that stress predisposes people to illness by influencing “stress hormones,” such as adrenal hormones, as well as catecholamines. These neuroendocrine actions, when produced by stress, are postulated to result in sequelae. Although it is clear that stress results in defined syndromes (such as PTSD) and enhances risk to other established medical conditions (such as myocardial infarction)—perhaps mediated through changes in intermediate factors (such as hemoconcentration and hypertension that are promoted with adrenergic arousal, or in the instance of PGW veterans, perhaps through enhancement of permeability of the blood-brain barrier, as discussed in Chapter Seven)—it is less well established (that is, not at all established) that stress might result in symptoms conforming to the frequency distribution seen in ill PGW veterans. However, if stress is postulated as an etiology for these symptoms, acting through acute neuroendocrine changes, then acute neuroendocrine actions precipitated by other etiologies, such as PB, would also need to be considered as possible sources of illness in veterans. PB exposure is estimated to have occurred in 250,000 to 300,000 PGW veterans (Brake, 1997). However, the effect of PB on hormones, including stress hormones, and the relation of these hormonal effects to chronic illness remain to be better elucidated.

CATECHOLAMINE EFFECTS

Catecholamines, such as epinephrine (adrenaline) and norepinephrine (noradrenaline), are part of the “fight or flight” response and part of the stress response. Central (brain) ACh stimulation appears to produce release of epinephrine. For example, physostigmine (a “carbamate,” like PB, that differs from PB by more readily crossing the blood-brain barrier and by being shorter acting) has been shown to produce marked increases in epinephrine levels and to a much lesser degree, norepinephrine levels—with profound increases in pulse rates and blood pressure (Janowsky, Risch, et al., 1985; Janowsky, Risch, et al., 1986). This effect occurs not through peripheral action of ACh on the adrenal medulla, where epinephrine is produced but through central (brain) effects. For example, if physostigmine is given together with agents that block
action of ACh in the body (the periphery), the effect still occurs: pronounced release of epinephrine along with increased pulse and blood pressure (with a dose of 0.022 mg/kg of physostigmine) (Kennedy, Janowsky, et al., 1984). As further evidence that the effect is central, neostigmine, a carbamate that like PB does not act centrally but only increases peripheral ACh levels, does not lead to similar increases in pulse and blood pressure in humans (Janowsky, Risch, et al., 1985). Nonetheless the effect is relevant to PB because in times of stress, PB may cross the blood brain barrier (see Chapter Seven, “Blood-Brain Barrier”), and PB may induce central effects, including those enhancing function of such “catecholamines” as epinephrine. (Importantly, since stress is associated with catecholamine release, PB may in turn augment the effects of stress.)

PAIN

Activating ACh receptors of the muscarinic type (for example, by administering the drug physostigmine, which is related to PB), stimulates release of the “endogenous opiate” or pain reducing substance termed β-endorphin (Risch, Janowsky, et al., 1981; Risch, Janowsky, et al., 1982a; Risch, Janowsky, et al., 1982b; Risch, Kalin, et al., 1983). Thus, downregulation of the muscarinic system would be expected to produce reduced release of β-endorphin, presumably associated with reduced analgesia—or heightened pain sensitivity. Thus, muscarinic downregulation could contribute to symptoms of pain in ill PGW veterans.

Perhaps more important, neuronal nicotinic downregulation could enhance symptoms of pain. Interest in the role of neuronal nicotinic ACh receptors in pain-processing has been rekindled by the discovery of epibatidine, a naturally occurring neuronal nicotinic ACh receptor agonist that has antipain activity more than 200 times greater than that of morphine (Donnelly-Roberts, Puttfarcken, et al., 1998). Work is ongoing to understand the role of these receptors in pain-signaling, and to develop agents selective for the neuronal nicotinic ACh receptors without binding to ganglionic and neuromuscular ACh receptors, to provide specific agents capable of pain relief (Barlocco, Cignarella, et al., 1998; Holladay, Wasicak, et al., 1998; Puttfarcken, Manelli, et al., 1997; Khan, Yaksh, et al., 1997; Damaj and Martin, 1996; Rao, Correa, et al., 1996; Bannon, Gunther, et al., 1995; Rupniak, Patel, et al., 1994; Damaj, Creasy, et al., 1994; Donnelly-Roberts, Puttfarcken, et al., 1998). Of note, development of tolerance to the antipain effects of epibatidine (downregulatory effects) has been found to show a different profile and characteristics compared to nicotine (Damaj and Martin, 1996). A nicotinic agent is currently being marketed for pain (by Abbott Laboratories).
Additional possible mechanisms of pain, mediated through effects on sleep and serotonin, are discussed below.

Of incidental note, Flinders Sensitive Line rats, bred to have cholinergic “hyperresponsiveness,” have higher pain thresholds than Flinders Resistant Line rats, bred to have low cholinergic responsiveness, when tested by the “jump-flinch” method (Pucilowski, Eichelman, et al., 1990). Depressives (who are generally more vulnerable to pain) show muscarinic supersensitivity to beta endorphin release (as well as to pituitary adrenocorticotropic hormone release) (Risch, Janowsky, et al., 1981); heightened sensitivity may accompany low baseline activity.

MOOD AND BEHAVIOR

Mood

A body of literature has linked ACh to mood, and both cholinergic and cholinergic-adrenergic models of mood disorders have been put forth (as have more complicated models which also incorporate other neurotransmitters) (Janowsky, El-Yousef, et al., 1974; Risch, Kalin, et al., 1981; Janowsky and Risch, 1984; Janowsky and Risch, 1986; Janowsky and Risch, 1987; Janowsky, Risch et al., 1988; Janowsky and Overstreet 1990; Janowsky, Overstreet, et al., 1994; Janowsky and Overstreet, 1995). Cholinergic agents have acute effects on mood, including induction of depression, anergia, and behavioral inhibition and suppression of manic symptoms when given alone or combined with other agents (El-Yousef, Janowsky, et al., 1973; Janowsky, El-Yousef, et al., 1973; Janowsky, Risch, et al., 1983). Cholinergic system “downregulation” would, then, not be expected to be prodepressive and could conceivably have an anti-depressive effect. Counterbalancing this in ill PGW veterans is the effect of chronic illness itself, which may be expected to favor the genesis of depression.

Evidence suggests that depressed individuals may have a sensitive muscarinic system, with increased vulnerability to cholinergic stimulation (possibly leading to increased vulnerability to affective and neuroendocrine disturbance), perhaps, it has been suggested, with muscarinic receptor “upregulation” (Janowsky, Risch, et al., 1980; Risch, Kalin, et al., 1981; Overstreet, Janowsky, et al., 1989; Gillin, Sutton, et al., 1991; Janowsky, Overstreet, et al., 1994). Thus, muscarinic stimulation (for instance with the drug arecoline, a muscarinic receptor agonist, or drug that mimics the effect of ACh on muscarinic receptors) produces greater effect on some tests (such as induction of REM sleep) in depressed subjects than in controls. However, other central cholinergic effects, such as the profound increase in serum epinephrine levels that normally occurs with such cholinergic-acting drugs as phsyostigmine, are relatively blunted rather than exaggerated in depressed individuals (Janowsky, Risch, et al., 1986).
The relation of these factors to illnesses in Gulf War veterans remains unclear. Many ill veterans have some depressive symptoms, but these may not occur more than would be expected in patients with a comparable degree of chronic illnesses. Ill veterans have been shown to have psychological profiles similar to those found in medical patients (Hom, Haley, et al., 1997). Mortality data indicate that, at least in the period 1991–1993, the suicide rate did not increase in PGW veterans; the adjusted mortality rate ratio from suicide was 0.94 for PGW veterans (n = 695,516) compared to other veterans (n = 746,291) (95 percent CI 0.79–1.12) (Kang and Bullman, 1996), suggesting that severe depression associated with suicide does not occur with increased frequency in PGW veterans, despite the significant pain and illness reported by many. Information on suicide rates for ill PGW veterans compared to (matched) medical patients has not been published. Thus, it is not known whether counter-depressive effects from ACh downregulation serve to partially offset factors disposing to depression and suicide, or whether rates of suicide are comparable to those with other medical illness.

**Behavioral Effects**

Limited behavioral information related to ACh is available from studies in animals. Rats selectively bred for increased cholinergic sensitivity (Flinders Sensitive Line), which seem to have low baseline ACh action but increased ACh responsiveness, are marked by reduced action in the face of stress. For instance, they performed poorly in a tone-cued two-way active avoidance task in comparison with the control Flinders Resistant Line of rat (Overstreet, Rezvani, et al., 1990). Such rats also exhibit a high degree of immobility in a forced swim test (Overstreet, Rezvani, et al., 1992). But Flinders Sensitive Line rats have increased cholinergic and serotonergic sensitivity, and the immobility in the swim test (assessed by the amount of time spent immobile in a five minute swim test) appears to segregate with the serotonergic rather than the cholinergic sensitivity. (These were assessed by looking at the “hypothermic” response (reduction in body temperature) to a serotonin activating drug (a chemical termed “8-OH-DPAT” or “8-hydroxy-2-(di-N-propylamino)tetratin,” which stimulates the serotonin 1A receptors); and to an ACh-activating drug (the anticholinesterase “oxotremorine” (0.2 mg/kg)) (Overstreet, Janowsky, et al., 1994).

**MEMORY AND COGNITIVE EFFECTS**

There is widespread evidence of effects of ACh on learning, memory, and attention (Levey, 1996; Barkai and Hasselmo, 1997; Levin, Torry, et al., 1997; Sarter and Bruno, 1997; Segal and Auerbach, 1997). Cholinergic underactivity is believed to underlie some human memory disorders, including Alzheimer's
disease (Davis and Davis, 1978). Nicotinic and muscarinic ACh receptors are important for maintaining optimal memory performance (Levey, 1996; Felix and Levin, 1997; Ohno, Kobayashi, et al., 1997; Vannucchi, Scali, et al., 1997). Nicotinic and muscarinic antagonists have been shown to impair performance in learning and memory tasks (Kohler, Ritters, et al., 1996; Felix and Levin, 1997; Harder, Baker, et al., 1998; Vannucchi, Scali, et al., 1997). Physostigmine can relieve the mental confusion produced by scopolamine (Ketchum, Sidell, et al., 1973; Granacher and Baldeassarini, 1975; Mohs, Davis, et al., 1979), and can reverse the memory deficit produced by anticholinergics like scopolamine (Drachman, 1977; Ghoneim and Mewaldt, 1977). Acetylcholinergic drugs like physostigmine and arecoline enhance learning and memory in animal studies (Robbins, McAlonan, et al., 1978) and in subjects with memory disorders, including Alzheimer’s (Peters and Levin, 1977; Agnoli, Martucci, et al., 1983; Christie, Sherin, et al., 1981). (Physostigmine, at the correct dose, may improve memory in those with certain memory problems but may be ineffective or perhaps harmful at too high or too low a dose (Peters and Levin, 1977; Mohs, Davis, et al., 1979).) The effects of ACh on cognitive function are diverse (Safer and Allen, 1971; Drachman and Leavitt, 1974; Ghoneim and Mewaldt, 1975; Drachman, 1977; Ghoneim and Mewaldt, 1977; Peterson, 1977; Mohs, Davis, et al., 1979; Beatty, Butters, et al., 1986) and difficult to summarize (Deutsch and Rogers, 1979; Sarter and Bruno, 1997) (though difficulties with some tasks involving visual attention, sensory integration, intermediate and long term memory, and spatial memory have been described) and generalizations from animal studies to human behavior have been described as not straightforward (Mohs, Davis, et al., 1979).

In short, ACh is vital for learning and memory, and disruption of ACh function—particularly low function but perhaps also too high function—impairs learning and retrieval of learned memories. Cognitive and memory impairment, which has been reported in some studies of ill PGW veterans (see Chapter Fourteen), might be an expected consequence if ACh dysregulation occurred following use of PB.

**DERMATOLOGIC FINDINGS: SKIN SYMPTOMS AND HAIR LOSS**

Skin symptoms (particularly rashes) have figured prominently in assessments of symptoms reported by ill PGW veterans. For example, rash represented the second most common complaint in an analysis of complaints of 17,248 ill or concerned veterans in the Veterans Affairs Persian Gulf Health Registry (as of June 1994), cited by 17 percent of such veterans (Persian Gulf Veterans Coordinating Board, 1995); and rash was reported by 35 percent of 125 reservists of the 123rd Army Reserve Command (De Fraites, Wanat, et al., 1992), and rash or dermatitis by 38 percent of 18,075 participants in DoD’s Comprehensive Clini-
cal Evaluation Program (CCEP) (DoD, 1996). There are many causes of rashes, including drugs, systemic infection, skin infection or disease, endocrine disease, autoimmune disease, and cancer. No reports have been uncovered in which PB was clearly linked to subsequent development of rashes. However, "central"-type nicotinic receptors have been identified in skin cells termed keratinocytes that may be involved in cell migration and adhesion (Grando, Horton, et al., 1995; Conti-Fine, Horton, et al., 1994). It is possible that if dysregulation of this system occurred, increased susceptibility to skin abnormalities presenting as rashes and other skin complaints would result. Some veterans have reported increased susceptibility to injury with "challenge," such as shaving or abrasion. This suggests that a test could be devised to measure skin susceptibility to injury with such challenge.

Many reports have surfaced that PGW veterans report dental problems, such as loose teeth, bleeding gums, or rapid dental decay (De Fraites, Wanat, et al., 1992; DoD, 1996; Gordon 1997). For instance, 47 percent of a group of 79 reservists from the 123rd ARCOM reported dental complaints; dental complaints were prominent in a list of symptoms by 33 ill British PGW veterans; and bleeding gums were reported by 8 percent of 18,075 CCEP participants (DoD, 1996). Whether problems with cell migration and adhesion resulting from AChE inhibition could contribute to bleeding gums or loose teeth remains a matter for future investigation.

Hair loss (alopecia) has also been reported among symptoms in ill PGW veterans from the United States (De Fraites, Wanat et al., 1992; DoD, 1996) and from the United Kingdom (Beale, 1994). For instance, hair loss was reported by 12 percent of 18,075 participants in the DoD’s CCEP (April 1996 report) (DoD, 1996). Hair loss may be caused by many conditions, ranging from endocrine disorders (such as hypothyroidism) to autoimmune diseases (such as lupus), drugs, infections (including leishmaniasis), and dermatological disorders. (“Telogen effluvium,” in which the normally asynchronous growth/death phase of follicles becomes synchronous, causing much of the hair to fall out at once, may occur with stress, pregnancy, and severe systemic illness (Adler, Lam, et al., 1994).) PB is among the drugs that have been reported to possibly cause alopecia. One report describes a 69-year-old woman who received 360 mg/day of PB (four times the PGW PB daily dose, in a patient with myasthenia gravis) in whom extensive generalized hair loss occurred several months after initiation of PB. PB was reinstituted one year later, and striking hair loss reoccurred after five weeks of use, with hair regrowth three months thereafter (Field, 1980).

Because many causes of rash and of hair loss are known, PB cannot be presumed to cause these symptoms in ill PGW veterans. However, because PB has been previously implicated in hair loss (albeit in one published case) and
because ACh may influence skin cell behavior, a role for PB in skin symptoms and reported hair loss cannot be excluded.

**DIARRHEA**

Diarrhea is commonly reported by ill PGW veterans. For instance, 20 percent of 18,075 participants in the DoD’s CCEP reported diarrhea, and for 2 percent it was the chief complaint. Diarrhea was reported as an acute symptom in patients who received PB. Indeed, muscarinic ACh symptoms, discussed in Chapter Three, include enhanced GI peristalsis. In addition, however, there is evidence of a connection to diarrhea for both high and low nicotinic ACh function.

Diarrhea, particularly in ulcerative colitis, has been treated successfully with nicotine (Griffel and Das, 1994; Guslandi and Tittobello, 1994; Pullan, Rhodes, et al., 1994; Silverstein, Lashner, et al., 1994; Nilsson, 1995; Rhodes and Thomas, 1995; Thomas, Rhodes, et al., 1995; Watson and Lewis, 1995; Birtwistle, 1996; Forbes, 1996; Guslandi and Tittobello, 1996; Pullan, 1996; Thomas, Rhodes, et al., 1996; Bonapace and Mays, 1997; Green, Thomas, et al., 1997; Sandborn, Tremaine, et al., 1997; Thomas, Rhodes, et al., 1998). Cigarette smoking is associated with protection against ulcerative colitis (with a risk ratio of 0.13 in one case control study, 95 percent confidence interval 0.05–0.38) (Silverstein, Lashner, et al., 1994), and cessation of nicotine has in some instances been linked to onset or exacerbation of this diarrheal condition (Birtwistle, 1995; Birtwistle, 1996), with those who smoked most heavily perhaps showing the greatest increase in risk (Birtwistle, 1996). Several factors reduce the impact of this finding—that nicotinic stimulation may ameliorate diarrhea, and nicotinic (ACh) withdrawal may produce diarrhea—as an explanation for diarrhea in ill PGW veterans. First, diarrhea in most ill PGW veterans has not been associated with inflammatory bowel disease. Although it is possible that low nicotinic function is responsible for diarrhea in ill veterans, and/or that nicotine will ameliorate symptoms of diarrhea, this has not been tested. Moreover nicotine may exacerbate rather than ameliorate symptoms in another inflammatory bowel disease termed Crohn’s disease (Bonapace and Mays, 1997; Thomas, Rhodes, et al., 1998).

**SLEEP**

This section briefly discusses the presence of sleep disorders in PGW veterans; the possible relationship between sleep abnormalities and neurochemical changes, specifically related to ACh and to serotonin; and the possible relationship between sleep disorder and other adverse outcomes in PGW veterans, including pain and death by unintentional injury.
Sleep Disorders in PGW Veterans

Sleep difficulties figure prominently in complaints of ill PGW veterans. In one early report, sleep abnormalities constituted one of the two most common complaints, along with headache (Newmark and Clayton, 1995); they were also the second most common (after fatigue) among a group of 79 reservists of 123rd ARCOM, endorsed by 57 percent of reservists (De Fraites, Wanat, et al., 1992) and the second most common specific diagnostic subcategory, after malaise and fatigue, among 6,517 CCEP participants with primary or secondary diagnoses of “Symptoms, Signs, and Ill-defined Conditions,” in whom sleep disturbances constituted a primary or secondary diagnosis of approximately 32 percent (Joseph, 1997). A more modest 5 percent of 17,248 ill or concerned veterans in the VA Persian Gulf Health registry (June 1994) reported sleep disturbances (Persian Gulf Veterans Coordinating Board, 1995). Ill PGW veterans from the United Kingdom also report sleep abnormalities (Beale, 1994).

Sleep apnea appears to be emerging as a prominent contributor to sleep abnormalities in ill PGW veterans. An evaluation of sleep disorders in 14 PGW veterans revealed that six had abnormal sleep studies, including three with obstructive sleep apnea, two with narcolepsy with abnormal multiple sleep latency test, and one with periodic movements of sleep with abnormal polysomnogram. (Narcolepsy is associated with decreased REM latency, consistent with abnormal ACh action—in this case, heightened ACh effect.) The most common diagnosis in this series, however, was “unspecified sleep disorder” (Newmark and Clayton, 1995). Another study has found a high prevalence of sleep apnea in self-referred PGW veterans with symptoms believed consistent with sleep disorder who were evaluated for sleep apnea; 15 of 46 evaluated, and 15 of 192 presenting met criteria for sleep apnea (Peacock, Morris, et al., 1997), suggesting that somewhere between 8 percent and 33 percent of these ill veterans had the disorder (since some who were not tested for the disorder might have tested positive). A study of consecutive CCEP participants found that sleep apnea was the primary diagnosis in 7.4 percent, and any diagnosis in an additional 5 percent of those PGW veterans who were registered (Roy, Koslowe, et al., 1998). (An additional 11.7 percent had any sleep problem as the primary diagnosis, and 19 percent as any diagnosis (Roy, Koslowe, et al., 1998).) Efforts are ongoing to characterize sleep disorders in a controlled, blinded fashion at the University of Texas Southwestern Medical Center at Dallas (Haley, 1998, citing work with R. Armitage and R. Hoffman).

ACh and Sleep Apnea. As noted, sleep apnea is the most common identified sleep abnormality in tested ill PGW veterans with sleep complaints (Peacock and Marris, 1997). A possible relation of sleep apnea to the ACh system (and ACh downregulation) is suggested by (mixed) evidence that nicotine may be useful in treating sleep apnea (Davila, Hurt, et al., 1994; Hein, Kirsten, et al.,
1995; Wali and Kryger, 1995; Wirth, 1995; Obermeyer and Benca, 1996; Schrand, 1996). Although this is consistent with the hypothesis in Chapter Thirteen that ACh downregulation as a possible sequela of PB administration may contribute to symptoms in ill PGW veterans, it does not constitute persuasive evidence for this hypothesis.

**ACh and Sleep: Other Information.** The acute effect of PB on the acetylcholinergic system is increased cholinergic activity. Long-term effects of PB (such as changes in the neuromuscular junction) have been demonstrated in animals. However, whether similar or unrelated long-term changes also occur in central muscarinic or nicotinic cholinergic synapses in humans is presently unknown, though animal data suggest that some similar effects may occur at central muscarinic sites. Elsewhere it is postulated that long term ACh dysregulation—and perhaps downregulation—may occur (see Chapter Twelve, “Neurotransmitter Dysregulation”). Sleep quality is believed to be affected by low brain ACh activity, and indeed the anticholinergic effects of antiparkinson drugs are believed to contribute to sleep quality disturbance in Parkinsonism.

ACh is known to be involved in regulation of REM sleep in particular. ACh (and agents that stimulate its action) increases REM sleep (Sitaram and Gillin, 1979; Kok, 1993). Effects of ACh on sleep have been shown by administering pilocarpine, a muscarinic “direct agonist” (agent that acts on muscarinic receptors directly to mimic the effects of ACh acting on those receptors). ACh-like muscarinic action in a double-blind study of 13 healthy males produced shortened REM sleep latency (time taken to first enter REM sleep, the phase of sleep in which dreaming occurs) and increased total REM time, REM percent (percent of time asleep that is spent in REM), and duration of the first REM period, and it reduced stage IV sleep and Delta sleep (Berkowitz, Sutton, et al., 1990). In this small, short study, subjective sleep experience was not affected. In another study, arecoline (another agent that stimulates the muscarinic type of ACh receptors) was shown to induce REM sleep in both depressed subjects and controls, in a dose-dependent fashion, when compared to placebo infusions; depressed patients entered REM more rapidly than control patients with a higher dose of arecoline, suggesting that depressed individuals might have a more sensitive muscarinic system (perhaps due to muscarinic receptor up-regulation) (Gillin, Sutton, et al., 1991).

Of note regarding possible long term effects of AChE inhibition: one study reports enhancement of REM more than one year after nerve agent exposure in the industrial setting (Bushfield and Duffy, 1982). (This appears to suggest ACh upregulation or activation for the REM system, rather than downregulation or depression with these agents.) Since the long-term effects of PB on the cholinergic system remain to be elucidated, whether such REM alterations occur long-term following PB (alone or with co-exposures, in selected individuals) remains
unknown. Short-term mild sleep deprivation, which ordinarily means selective REM deprivation because REM is more prevalent later in the course of sleep, produces mood elevation. Indeed, some antidepressants are thought to exert part of their action by shortening the duration of REM. Alteration of sleep architecture in the form of increased REM might be expected to lead to relative dysphoria.

The finding of prolonged increased duration of REM observed following exposure to soman suggests that, at least with that OP nerve agent in primates, long-lasting enhanced rather than reduced cholinergic function occurs in REM regulation, more consistent with the concept of cholinergic dysregulation, rather than exclusively downregulation. It is not known whether similar effects, no effects, or opposite effects (consistent with downregulation) would occur following exposure to PB—alone or in combination with other exposures—if it gains access to the brain. Cholinergic downregulation would be expected to result in increased REM latency and perhaps reduced REM duration. Ongoing sleep studies are evaluating sleep, including REM, in ill PGW veterans and controls, and results of these studies will allow these possibilities to be narrowed down (Haley, 1998b, 1998c). (The complexities of such evaluations are reinforced by the findings noted previously: effects on ACh function from AChE inhibitors may differ from one brain area to another, perhaps reflecting different properties of the resident ACh receptors; thus it is conceivable that lasting effects of AChE inhibitors on sleep regulation (if any) need not parallel effects of AChE inhibitors on other functions regulated by different brain areas or involving different classes of ACh receptors.)

**Serotonin and Sleep.** Sleep and serotonin may each influence the other. Serotonin may influence sleep because serotonin is the precursor of melatonin (Hardman, Limbird, et al., 1996), which is involved in regulation of sleep. Sleep may in turn influence serotonin because serotonin is preferentially produced during stage IV sleep (Duna and Wilke, 1993).

PB may affect sleep, as noted previously, perhaps through enhancement of REM. If total sleep time is preserved, REM enhancement may lead to absolute or relative reduction in stage IV sleep, which could influence serotonin production. However, it remains unsupported that long-term changes in REM or stage IV sleep occur following short-term administration of PB.

As noted previously, PB appears to bind to the serotonin binding site on the ACh receptor, offering a possible avenue for disruption of serotonergic mechanisms from PB not mediated through sleep (see Chapter Three). (For example, if altered regulation of ACh receptors—increase or decrease—takes place, then a commensurate increase or decrease in serotonin binding capability results, which may, though feedback mechanisms, lead to further changes in regulation.
of the serotonin system.) Whether such disruption occurs with PB, or in Gulf War veterans, has not been established.

In war-related PTSD, elevated awakening thresholds in sleep stages III and IV have been demonstrated, although overall sleep data were within normal limits (Dagan, Lavie, et al., 1991), and no evidence of change was found in the proportion of slow wave sleep. This finding may serve as a caution that sleep that appears “normal” by crude sleep measures may in fact differ in more subtle characteristics, perhaps with neurochemical correlates (as a cause or effect).

Possible Sequelae of Sleep Alteration in PGW Veterans

Sleep, Pain, and Fibromyalgia. Fibromyalgia is a condition of chronic pain entailing widespread pain; and pain with finger pressure over at least 11 of 18 designated “tender point” sites. Fibromyalgia occurs predominantly in females in the civilian population (Bennett, 1995), but fibromyalgia and similar pain syndromes are common in ill PGW veterans (about 93 percent of PGW veterans are male). For example, muscle and/or joint pain was the fourth most common symptom among 7,248 ill or concerned veterans in the VA Persian Gulf Health Registry, June 1994, represented in 14 percent of registrants (Persian Gulf Veterans Coordinating Board, 1995). Diseases of the musculoskeletal system and connective tissue constituted the most common primary diagnosis among a group of 20,000 CCEP Gulf War participants, occurring as a primary diagnosis in 19 percent and as a secondary diagnosis in 30 percent (Joseph, 1997). “Joint pains” were reported by 54 percent of reservists of the 123rd ARCOM (n = 79) (De Fraites, Wanat, et al., 1992)—tied for the third most common complaint. In a series of consecutive PGW veterans referred to a CCEP, rheumatological consultation was the most common elective subspecialty referral (56 percent); among those referred, 59 percent had soft tissue syndromes, in which fibromyalgia was prominent (17 percent) (Grady, Carpenter, et al., 1998).

In light of the high prevalence of soft tissue pain in ill veterans and the high prevalence of sleep disorders, the known relation between sleep disorder and fibromyalgia merits discussion.

Non-REM stage IV sleep is disrupted in fibromyalgia (Saskin, Moldofsky, et al., 1986; Bennett, 1995). If stage IV sleep is disrupted intentionally in normal controls, fibrotic symptoms develop (symptoms akin to those in patients with fibromyalgia) (Duna and Wilke, 1993). Although periodic leg movements are the most common sleep disorder diagnosis associated with fibromyalgia, sleep apnea is a relatively common finding in men with fibromyalgia (Bennett, 1995).

Serotonin is preferentially produced in stage IV sleep, and these patients are reported to have reduced levels of serum serotonin and CSF 5-HIAA, a
metabolite of serotonin (Duna and Wilke, 1993). Moreover, if tricyclic antidepressants are given, which raise serotonin levels and enhance stage IV sleep, symptoms abate (Duna and Wilke, 1993). It is thought that reduced serotonin from stage IV sleep deprivation may lead to lowered pain thresholds and “activation” of latent tender points by one of two possible mechanisms: attenuating the pain-modulating effects of endogenous opiates called “endorphins” (Duna and Wilke, 1993; Vaerøy, Helle, et al., 1988a); or altering the function of a neurotransmitter involved in signaling pain, called “substance P,” so that sensory stimuli are more likely to be interpreted as pain (Duna and Wilke, 1993; Murphy and Zelman, 1987). Serotonin deficiency may explain the elevated CSF levels of substance P found in patients with fibromyalgia (Vaerøy, Helle, et al., 1988b; Duna and Wilke, 1993). (In turn, substance P may enhance cholinergic receptor desensitization, and is viewed by some as an inhibitory modulator at nicotinic cholinergic sites (O’Neill, 1981; Stallcup and Patrick, 1980) (see chapters on neuromuscular junction and neurotransmitter dysregulation)); thus stage IV sleep deprivation, if present, could theoretically potentiate any effects of cholinergic downregulation.

**Sleep and Accidents.** Sleep disruption has been a prominent symptom in PGW veterans in some reports (noted previously). Increased deaths from motor vehicle accidents have also been reported in ill PGW veterans (Kang and Bullman, 1996).

Motor vehicle accidents are strongly associated with sleep deprivation, circadian disruption, and sleep disorders (Gold, Rogacz, et al., 1992; Maycock, 1996; Findley, Weiss, et al., 1991). The risk has been particularly well studied for sleep apnea, which as noted above may be increased in ill PGW veterans. Studies report from a twofold to a more than sevenfold increased risk in all or (particularly) single car accidents, or in those for which subjects were at fault (Haraldsson, Carenfeldt, et al., 1995; Findley, Unverzagt, et al., 1988; Stoohs, Guilleminault, et al., 1994); one reports a twelvefold higher risk of single-car accidents adjusted for miles driven (Haraldsson, Carenfeldt, et al., 1990). The risk may rise with increasing severity of sleep apnea (Findley, Fabrizio, et al., 1989). Moreover, treatments (such as nasal continuous positive airway pressure, or uvulopalatopharyngoplasty) that reduce symptoms and signs of obstructive form of sleep apnea also have been reported to significantly reduce the rate of accidents in obstructive sleep apnea sufferers (Flemons and Tsai, 1997; Cassel, Ploch, et al., 1996; Haraldsson, Carenfeldt, et al., 1995). (Such treatment also benefits mood and cognitive effects associated with sleep apnea (Flemons and Tsai, 1997).) Special driving simulation tests may predict which patients with sleep disorders are at increased risk for automotive accidents (Findley, Unverzagt, et al., 1995), and such tests could be considered for use in ill PGW veterans with identified sleep abnormalities.
Increased injury deaths have been reported in PGW veterans (see “Violent Death” section in this chapter). While other mechanisms may be postulated for this increase in violent death, the presence of widespread reports of sleep abnormalities requires that sleep disruption be investigated as a contributing factor.

**Summary.** Whether sleep disruption reported by PGW veterans relates to use of PB (alone or with anticholinesterase coexposures) remains unknown. If long-term cholinergic or serotonergic changes are produced by PB, a matter that remains unresolved, then alterations in REM sleep or stage IV sleep, respectively, may be produced. Sleep apnea, which has been the most prominent specific sleep disorder in ill PGW veterans, has been associated with fibromyalgia and could contribute to symptoms of pain reported by PGW veterans. Whether or not sleep abnormalities relate to prior use of PB, both subjective and objective sleep abnormalities, including particularly sleep apnea, have been documented in a substantial fraction of tested PGW veterans reporting sleep problems. Because sleep disorders (particularly sleep apnea) and sleepiness have been strongly linked to increased risk of automotive accidents in several studies, reported and identified sleep disorders in ill PGW veterans are a possible or even likely contributor to the increased rate of accidental death observed in PGW veterans (see section on “Violent Death”).

Because sleep disruption is widely reported in ill PGW veterans and can have serious sequelae including pain and death from injury, additional effort should be made to characterize the sleep abnormalities of ill PGW veterans, to determine the nature of the abnormalities and if possible devise effective treatments. Consideration could be given to a trial of nicotinic agonists for ill PGW veterans with sleep apnea.

**TERATOGENICITY AND DEVELOPMENTAL EFFECTS**

**Birth Defects in Children of PGW Veterans**

Epidemiological studies indicate that children of PGW veterans are no more likely than controls to exhibit serious birth defects (Cowan 1997). However, risk of birth defects was increased in children of deployed women (borderline significance: RR 1.12, 95 percent CI 1.00–1.25), although the increase was no longer significant after adjustment for race/ethnicity, marital status, and branch of service (Cowan, De Fraites, et al., 1997). This study has been criticized on the grounds that by confining evaluation to births of active-duty personnel in military hospitals, those with illness (who would have been preferentially separated from the military) would have been preferentially excluded (Haley, 1998b, 1998c). If exposures of some kind associated with Gulf
War service are responsible for illness, and also for birth defects, then the very population at risk for birth defects would have been excluded from evaluation.

One study reported that children of 52 National Guardsmen from two Mississippi National Guard units deployed to the Persian Gulf had a frequency of minor and major birth defects, premature births, low birth weight, and other health effects (based on examination of medical records of 54 of 55 children born to those veterans) supposedly similar to that in the U.S. general population (Penman, Tarver, et al., 1996). However, this conclusion was rendered from this small sample in the absence of statistical analysis.

One study examined the presence of Goldenhar’s syndrome (or “oculoauricular vertebral dysplasia”), one of the birth defects that had been described in the popular press) among 34,069 infants of Gulf War veterans and 41,345 infants of non–PGW deployed personnel conceived after return from the Gulf (or after December 31, 1990, for non–PGW deployed) born in military hospitals before October 1, 1993, from parents still on active duty (Araneta, Moore, et al., 1997). Goldenhar’s syndrome is characterized by abnormal facies (facial appearance), including ear abnormalities (microtia, anotia, or preauricular tags), asymmetry or hypoplasia of the face or mandible, unilateral cysts on the eyeballs (epibulbar dermoids), defects (“colobomas”) of the upper lids, and lateral facial clefts, as well as vertebral anomalies. Medical records, subsequent hospital admissions, and genetic evaluations of all infants diagnosed with anomalies of the face or skull, or those with defects associated with Goldenhar’s syndrome, were examined by two pediatricians blinded to Gulf War status. A threefold excess risk of Goldenhar’s syndrome was derived from small numbers of affected infants and was not statistically significant (RR 3.03, 95 percent CI 0.63–20.57). Again, by confining evaluation to offspring of active-duty personnel born in military hospitals, it is possible that those at greatest risk for birth defects may have been preferentially excluded from analysis, although it cannot be presumed that inclusion of those individuals would not necessarily buttress the case for increased incidence of Goldenhar’s syndrome.

An ongoing VA Cooperative Study of PGW veterans and their families plans to look at severe birth defects only (Murphy, Kang, et al., 1998). Confinement to severe birth defects necessarily restricts the number of endpoints evaluated, possibly reducing the power to detect an effect; moreover, since prior study suggested the possibility of increase in all birth defects but not severe birth defects in offspring of female veterans (Cowan, De Fraites, et al., 1997), this plan precludes analysis of those defects that existing evidence suggests may be increased.

A study is under way evaluating birth defects among children of British Gulf War veterans (Doyle, Roman, et al., 1997).
PB: Fetal and Developmental Effects

As noted in Chapter Three ("Characteristics of PB"), PB may cross the placenta. Controlled trials cannot be done in humans to evaluate the effect of PB on fetal development. Controlled studies can be done in animals, and observational studies are possible in humans.

PB use during pregnancy in myasthenics has been postulated as a contributor to neonatal myasthenia (Blackhall, Buckley, et al., 1969), a condition involving transient weakness of the infant seldom persisting beyond six weeks after birth; however, the condition may primarily arise from maternal antibodies to the ACh receptor circulating in the fetus. Reports have not been identified indicating teratogenicity from PB administration in myasthenics, despite the fact that substantially higher doses of PB (e.g., 600 mg/ d; or 6.7 times the PGW dose) are given in this population, and treatment occurs for a more prolonged period. This situation provides additional reassurance that gross fetal abnormalities are not common with PB administration during pregnancy.

Animal studies evaluating "teratogenic" or developmental effects of PB have been done in rats and chickens. In rats, developmental concerns of PB occur for adult male offspring of females exposed while pregnant, although some of these data derive from studies employing neonatal rather than in utero PB delivery: Neonatal delivery of PB (2 µg/day for four days, then 10 µg/day for 10 days) was found to permanently increase male sexual behavior in those rats that exhibited even slight hypoplasia of seminal vesicles in neonatal life, reinforcing the notion that changes of neurotransmitter concentrations and/or turnover rates induced by psychotropic drugs can affect sex-specific brain differentiation (Dorner and Hinz, 1978). Moreover, adult male rats treated neonatally with PB (2µg/day for four days—or 0.03 GWE—then 10 µg/day for 10 days in 20–25 g rats—or 0.31 Gulf War daily doses) showed a slight decrease in the noradrenaline concentration in the hypothalamus. It was concluded that PB (and other psychotropic substances) may exert "teratogenetic" effects, which are mediated, at least in part, by unphysiologic concentrations and/or turnover rates of neurotransmitters during brain differentiation (Dorner, Hecht, et al., 1976; Dorner, Staudt, et al., 1977).

Studies in chicken embryos with cholinomimetic agents, including neostigmine (in doses from 0.1 to 0.6 mg), PB (in doses of 10 mg/egg or more), physostigmine, carbachol, decamethonium, and others found that all these compounds led to abnormalities of the cervical vertebrae, or the whole vertebral column (Landauer, 1975). Hypoplasia of leg muscles occurred with lower incidence. A high degree of synergism was seen when two cholinomimetic compounds were used in combination (Landauer, 1975). PB results were not reported in detail, but PB showed among the stronger effects, exceeding those
of neostigmine, and accounting for short crooked necks and muscular hypoplasia of the legs. Physostigmine produced additional abnormalities including syndactylism (webbed digits), micromelia (shortened limbs), and abnormalities of the “visceral skeleton” and of the eyelids (Landauer, 1975).

Other studies looking at gross survival and morphological findings in rodents do not provide strong support for teratogenic concerns. When female rats were given 50–60 times the human dose of PB (on a mg/kg basis) in their drinking water on days 6–16 of pregnancy, there was reportedly no adverse effect on pregnancy, litter size, resorption rate, malformation rate, or fetal development (Wetherell, 1992). Although amount of PB actually consumed was not reported, the highest dose reportedly led to marked signs of toxicity and initial weight loss. Of course, this does not preclude effects of PB during developmental periods outside days 6–16 of pregnancy. When female rats were given PB from 15 days before mating to day 20 of gestation, no adverse effects were identified on mating, fertility, resorption rate, litter size, gestation length, malformation rate, or skeletal development. There was a slight reduction in postnatal pup weight gain but no effect on pup survival or time of developmental landmarks or visual or auditory function. When male rats were given PB in their drinking water for 18 weeks, mating performance and fertility were reportedly not affected. When female rabbits were given 93 times the human dose (on a mg/kg basis) of PB in their drinking water (no data were given showing how much was actually consumed), PB was stated to produce no “treatment related effect” on pregnancy, resorption rate, or malformation rate (Wetherell, 1992). It was concluded that these agents were safe for use in men and in women of childbearing age.

Clearly, teratogenic effects may be species- and dose-dependent. The data presented here suggest that monitoring for effects is appropriate, particularly for the small number of female veterans who received PB while pregnant. Moreover, while identification of gross abnormalities may occur by inspection, identification of delayed behavioral effects may not be readily apparent at birth or even in later life, without suspicion and careful investigation. Of note: alteration in the ratio of male to female births (a relative reduction in males—presumed to be opposite to the effect that might be expected from gender-selective abortion) has been reported in several industrial countries and is proposed as a possible “sentinel health indicator” (Davis, 1998). If this is correct, this ratio most likely relates to exposures distinct from AChE inhibition. Nonetheless, consideration could be given to comparing the sex ratio of children born to PGW veterans to the ratio in controls, or in the general population (considering female veterans and controls separately).
PREGNANCY AND PGW

Pregnant women were not knowingly deployed to the PGW, though some women were later determined to have been pregnant at or during the time of deployment. Pregnancy was cited as a reason for women personnel to consult medical personnel prior to taking PB (see Chapter Four, “PB Use in the Persian Gulf War”). Children of these women would seem to be those at greatest risk of manifesting effects, if any, resulting from maternal PB exposure. The number of children possibly affected by this exposure would be small, and these children should be easily identified.

If changes in brain development do result from in utero exposures, resulting behavioral alterations might not be identified until years after birth, and then perhaps only with careful study and may not be attributed to PB. An analogous experience can be cited from women in the 1950s who were given progestins to prevent miscarriage—daughters of these women reportedly exhibit characteristic differences in sex-typical behavior that begin to be evident in childhood. In addition, some but not all experienced minor birth defects, such as clitoromegaly. It is unclear whether testing for behavioral differences would have been performed, and behavioral differences identified, in the absence of identified physical changes at birth in a subset.

Consideration could be given to performing case control studies of behavior and cognition in children of female myasthenics who took PB while pregnant. While children of female PGW veterans who took PB while pregnant could also be evaluated, the sample would be quite small. These studies would have limited impact on future military use, because pregnant women are not knowingly deployed.

VIOLENT DEATH (DEATH FROM UNINTENTIONAL INJURY)

While illness mortality has not been elevated in PGW veterans (adjusted mortality rate ratio for “disease related causes” of death in PGW veterans compared to other veterans was 0.88, with a 95 percent confidence interval (CI) of 0.77–1.02, adjusted for age, race, sex, branch of service, and type of unit), mortality from unintentional injury was increased in deployed compared to nondeployed veterans (Kang and Bullman, 1996). Mortality rate ratios for all external causes were 1.17 (95 percent CI 1.08–1.27); for all accidents 1.25 (1.13–1.39); and for motor vehicle accidents 1.31 (1.14–1.49) (Kang and Bullman, 1996). This increase in accidental death was sufficient to produce increased overall mortality in PGW veterans compared to veteran controls. Despite no increase in illness mortality, and indeed a trend toward reduction, the adjusted overall mortality rate ratio showed a 9 percent increase, and this increase was statistically significant (95 percent confidence interval 1.01–1.16) (Kang and Bullman, 1996).
In the year from August 1, 1990 through July 31, 1991, more than half of all non-battle deaths in PGW era active duty personnel were from unintentional injury; and more than half of these from motor vehicle accidents (Committee to Review the Health Consequences of Service During the Persian Gulf War; IOM, 1996). The relative risk, or risk in deployed veterans divided by risk in non-deployed veterans, was 1.54, indicating a 50 percent increase in Gulf War deployed personnel; the 95 percent confidence interval was 1.32–1.77, signifying that, with 95 percent confidence, the smallest true increase supported by the statistics was 32 percent (Committee to Review the Health Consequences of Service During the Persian Gulf War; IOM, 1996). As noted above, mortality follow-up of PGW veterans supports a continued increase in the rate of injury death in the postwar period (Kang and Bullman, 1995; Kang and Bullman, 1996).

There are many possible causes of accidental death in PGW veterans, including some that may relate to PB. Several possible PB-related alternatives include cholinergic overactivity; sleep abnormalities, dependent on or independent of cholinergic changes; and possibly changes in serotonergic activity. Substance abuse relates strongly to violent and accidental death. However, Department of Veterans Affairs studies shortly after demobilization indicated a relatively low prevalence of substance abuse of 1.7 percent (Committee to Review the Health Consequences of Service During the Persian Gulf War; IOM, 1996); no comparison numbers were provided.

**Cholinergic Activation**

Many men were said to report enhanced aggression after taking nerve agent pretreatment sets with PB (Currie, 1995). Consistent with an AChE-inhibiting mechanism for this effect, aggressive behaviors have also been reported following exposure to other cholinesterase inhibitors including OPs and carbamates (Devinsky, Kernan, et al., 1992). Aggression by a person is related to nonillness mortality (death from injury or violence) in that person. Violent deaths, including homicide, suicide, and accident are interrelated and tracked together as nonillness mortality in international studies (Holinger and Klemen, 1982). Death by these three modes are linked etiologically through low serotonin (see below), through alcohol, through substance abuse, and through psychiatric disease (in which an apparent “accidental death” may in some instances represent a covert suicide or occur as a consequence of grossly impaired judgment). However, in PGW veterans, these causes of violent death are dissociated. Rates of death from suicide and homicide do not appear to have increased commensurately with accidental death in the PGW veteran population (see below). Other mechanisms may more selectively affect accidental death.
Sleep Deprivation

Sleep deprivation and sleep disorders are known to be strongly associated with death by motor vehicle accident. Many veterans report sleep abnormalities; therefore this etiology should be strongly considered (see section on “Sleep,” above).

Reduced Serotonin

Natively low or experimentally lowered serotonin has been strongly associated with impulsive violent behaviors, risk-taking behaviors, and violent outcomes in many studies in humans and animals (Grant, Cocsina, et al., 1973; Kulkarni, 1968; Kantak, Hegestrand, et al., 1980; Miczek and Donat, 1989; Miczek, Mos, et al., 1989; Olivier, Mos, et al., 1989; Kostowski and Valzelli, 1974; Åsberg, 1994; Eichelmann, 1979; Coccaro, 1989; Coccaro, Siever, et al., 1989; Brown, Goodwin, et al., 1982; Brown, Ebert, et al., 1982; Brown, Goodwin, et al., 1979; Brown and Goodwin, 1986a; Brown and Goodwin, 1986b). However in humans, reduced serotonin is strongly associated with suicide in particular, an effect that cuts across psychiatric diagnoses (whether persons have unipolar depression, personality disorder, or schizophrenia, it is the low serotonin subgroup that is at greatest risk for suicide attempts (Grant, Cocsina, et al., 1973; Kulkarni, 1968; Kantak, Hegestrand, et al., 1980; Miczek and Donat, 1989; Miczek, Mos, et al., 1989; Olivier, Mos, et al., 1989; Kostowski and Valzelli, 1974; Åsberg, 1994; Eichelmann, 1979). While suicide was a significant cause of death in personnel who were on active duty in the PGW and elsewhere (with 216 deaths from suicide among the 1,622 total nonbattle deaths from August 1, 1990, to July 31, 1991), the relative risk for suicide among PGW-deployed compared to non-PGW deployed veterans was 0.34 during this period (95 percent CI 0.16–0.63) (Writer, De Fraites, et al., 1996), signifying a markedly reduced rate of death from suicide during the war. Data from after the war, derived from a comprehensive study of mortality among all 695,516 personnel who served in the Persian Gulf from August 1990 to April 1991, compared to 746,291 personnel who served elsewhere during the same time, found the postwar suicide rate in men to be comparable in PGW veterans to that in controls; women showed a nonsignificant trend toward increased suicide and homicide (Kang and Bullman, 1995; Kang and Bullman, 1996). The mortality rates for PGW veterans versus controls in 1991–1993, based on death certificate information, adjusted for age, race, sex, branch of service, and type of unit were as follows: for suicide 0.94 (0.79–1.12) and for homicide 0.85 (0.67–1.08). Suicide and homicide were not increased, while all accidents and motor vehicle accidents were. These data do not support either a low serotonin state or substance abuse as major contributors to death from injury in PGW veterans and add credence to an alternative mechanism involving impaired neurocognitive function, such as
impaired concentration or decisionmaking, whether due to sleep disruption, altered neurochemistry from PGW exposures (perhaps including PB), or other sources.2

Increased accidental deaths have been reported following other conflicts, including World War II prisoners of war (Eberly and Engdahl, 1991); and Vietnam veterans (Watanabe and Kang, 1995). In Vietnam veterans, the relative risk of death from “external causes” was 1.21 (95 percent CI 1.0–1.47) (Watanabe and Kang, 1995). Greatly elevated rates of PTSD were noted particularly among prisoners of war. Associated sleep and concentration disruption from PTSD and perhaps substance abuse may have been etiologic following these conflicts.

Both similar and different exposures occurred in the different conflicts, and similar and different etiologies for violent death may be at play. In contrast to the PGW, increased incidence of suicide has been reported for Vietnam veterans (Watanabe and Kang, 1995); this adds support to etiologies of violent death that include accident and suicide (as many do), rather than etiologies more specific for accidents. Thus, PTSD, alcohol and substance abuse, and serotonin dysregulation may play a more significant role in the increased violent deaths observed in Vietnam veterans, while these etiologies are less strongly supported for ill PGW veterans.

DEFINING CASES AND CONTROLS FOR RESEARCH ON ILLNESSES IN PGW VETERANS

Because there was no particular location in previous chapters for discussion of how Gulf War veteran cohorts should be defined for the purpose of clinical studies (which may be different from definitions for other purposes), such a discussion is included here. Subjects selected as representing ill PGW veterans for case-control studies should ideally be those with more characteristic and perhaps more severe symptoms: use of all PGW veterans, all who chose to enter registries, or all who report any illness will dilute the sample and complicate the ability to detect true associations. Moreover, use as healthy controls of all veterans who do not enter registries is particularly problematic, because preliminary work suggests that many who have not elected to participate in registries may experience similar symptoms. Some groups are beginning to generate case definitions of illness in PGW veterans for the purpose of studies they are conducting. One group uses degree of compatibility with factor-analysis defined syndromes in ill PGW veterans (Haley, Kurt, et al., 1997) for this purpose (Haley, 1997).

Comment: Data emerging after this writing suggest an increase in suicide may be present, with a risk ratio of 1.53; homicides, however, were not increased (RR 0.85).
1998); this permits assessment of how “typical” symptoms are, at least according to the factor-analytic standard.

The soundness of the factor-analytic strategy would be strongly enhanced if the results were replicated using cross validation, or if other techniques, such as unsupervised neural networks, were shown to categorize subjects similarly. However, while this standard can be questioned, case assignment error resulting from use of this strategy would likely produce bias toward the null and would not be expected to engender spurious positive findings.

“Healthy” controls should be selected to have none of the characteristic symptoms—no unusual fatigue, headache, joint and muscle pains, or sleep disturbance and no new onset of diarrhea, rash, mood alteration, headache, chemical sensitivities, or difficulty concentrating. A separate group of “unhealthy” controls could be defined in whom symptoms are present but quite distinct from those of persons with “characteristic” illness. The goal is to separate groups at extremes of symptomatology, just as studies of cardiovascular risk factors may compare those in the highest quintile on some factor—either exposure or outcome—to those in the lowest quintile, to increase potential for assessment of the link between exposure and illness. The criteria employed for the purpose of study are expressly intended to identify the more typical cases (thus, those most likely to be part of a coherent syndrome in which risk factors may be identified), and while they may not be selected as cases, it should not be presumed that others with atypical or lesser symptoms are necessarily “free” of illness.

**SCIENTIFIC RECOMMENDATIONS**

- Sleep studies should be considered in PGW veterans who complain of fatigue; evaluation for sleep apnea should be included.
- Sleep studies in PGW veterans who complain of sleep difficulties or fatigue should specifically examine sleep parameters known to be related to cholinergic dysfunction, such as REM sleep latency and duration.
- Sleep studies in PGW veterans who complain of sleep difficulties should specifically evaluate stage IV sleep patterns, including duration, timing (including latency), proportion of total sleep, and awakening.
- Consideration should be given to evaluating other indices of serotonergic function, including peripheral and central measures.
- Investigation of circadian functioning (e.g., of neurotransmitter and hormone levels and responsivity, and such autonomic indices as temperature and blood pressure) should be done in ill PGW veterans and controls.
• Consideration should be given to evaluating automobile accident risk in PGW veterans with fatigue and/or identified sleep disorders, using driving simulation tests that have been shown to correlate with accident risk in sleep-disordered drivers.

• Additional work should be done to evaluate contributors to death by unintentional injury. Persons with hospitalization for unintentional injury may be compared to controls on measures that may relate to injury risk. Candidate measures include subjective fatigue; subjective and objective sleep measures; sensitive neuropsychiatric measures, such as those involving attention, psychomotor speed, or visual function; and perhaps measures of cholinergic and possibly serotonergic function and substance abuse.

• Strong emphasis should be placed on research performed to identify specific objective abnormalities that may characterize subsets of ill PGW veterans. This may include clinical tests of vestibular function, eye movement, neuropsychiatric function, autonomic function, such laboratory tests as tests of cytokine function or porphyrin metabolism, and such imaging tests as tests of regional blood flow. Identification of such abnormalities in ill veterans will be essential to pursuing tests of etiology in animal models. Specifically, different candidate exposures can be tested for production of similar objective findings, thus abetting the process of identifying causative or contributory exposures. This recommendation is applicable to study of etiologies and contributory factors discussed in all chapters.

• Clinical case-control studies should endeavor to define cases and controls in a manner that would permit assessment of a link to risk factors, if such a link is present. Thus, cases should consist of those with more “characteristic” clusters of symptoms; healthy non-PGW (and perhaps PGW) controls should consist of those who lack both characteristic and equivocal or less typical symptoms, and unhealthy non-PGW (and perhaps PGW) controls can be defined as those in whom health symptoms are present, but do not include characteristic symptoms, or equivocal or less typical symptoms, of ill PGW veterans. As scientific advances are made, objective correlates of illnesses in PGW veterans may be identified. Subsequent case definitions may then be modified to incorporate those findings. There is ample evidence that ill PGW veterans are ill from more than one cause. (A small number, for example, have been found to have viscerotropic leishmaniasis, but this is unlikely to account for illness in most ill veterans.) Strategies for clustering data should be considered for defining symptom clusters, in an effort not to confute findings by mixing different illnesses in one “case” definition. (Note: since this report was originally sent for review, additional efforts toward such case definitions have been generated, notably that by the CDC (Fukuda, Nisenbaum, et al., 1998).)
SUMMARY ANALYSIS

This chapter discusses an assortment of etiologies and hypotheses relating PB to physiological function or outcomes. These hypotheses are evaluated in lesser detail than in other sections, and summary analyses for each area presented in this chapter are not provided.