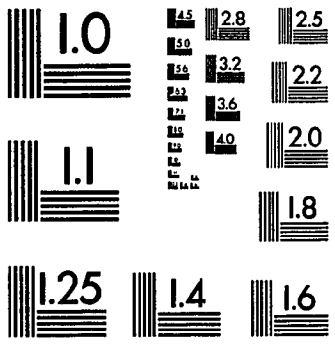

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AGE-RELATED CHANGES IN STRESS AND OPIATE RESPONSES IN RATS

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AGE-RELATED CHANGES IN STRESS AND OPIATE RESPONSES IN RATS

by

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Abstract

AGE-RELATED CHANGES IN STRESS AND OPIATE RESPONSES IN RATS

by

Elisse Kramer

Advisor: Professor Richard Bodnar

Aging is accompanied by impaired regulation of central nervous system responses that play a role in homeostatic functioning and adaptive coping responses to stress. Consequently, the present study assessed whether analgesic and other adaptive responses following morphine administration and acute exposure to two physiological stressors, 2-deoxy-D-glucose (2DG) and cold water swims (CWS), varied as a function of age. Separate age groups of female rats (4, 9, 14, 19 and 24 months) were tested in the three experimental manipulations. In the first experiment, 2DG analgesia (0, 50, 250, 450, 650 mg/kg) was assessed at 3 post-injection intervals on both the tail-flick and jump tests. Subsequently, 2DG (650, 1200 mg/kg) hyperphagia was assessed. Significant decreases in 2DG analgesia were found on both pain tests with increases in age. 2DG hyperphagia was present in the 4 and 9-month groups, absent in the 14-month group, and hypophagic rather than hyperphagic in the 19 and 24-month groups. In the second experiment, CWS analgesia and hypothermia were assessed at 3 intervals following a range of water temperatures (no swim, 21, 15, 8, 2^{o}C). An abrupt decrease in CWS analgesia occurred in the 24-month group on the tail flick test, but a more gradual decline was shown by the 14, 19 and 24-month groups on the jump test. Although all groups displayed a temperature-dependent hypothermia, the three older age groups showed a

greater hypothermic response. In the third experiment, morphine analgesia and hyperthermia (0.0, 1.0, 2.5, 5.0, 10.0 mg/kg) were assessed at 4 post-injection intervals. Morphine analgesia on the tail-flick test was delayed but persisted for a longer period of time in the 14, 19 and 24-month groups, whereas consistent reductions were observed on the jump test. In contrast, no age-related differences were found in morphine-induced hypothermia. Thus, with increase in age the analgesic and stress response systems evoked by glucoprivation, CWS, and morphine are differentially affected. Although reduced analgesia was generally observed after all manipulatons, the differential patterns of decline following the various manipulations as well as their dissociation from other physiological measures, suggest that aging in rodents is associated with specific rather than generalized changes in adaptive responses to stress.

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AGE-RELATED CHANGES IN STRESS AND OPIATE RESPONSES IN RATS

Aging is accompanied by impaired regulation of autonomic and central nervous system responses that are important to homeostatic functioning and adaptation to stress (Selye and Tuchweber, 1976). These changes have been extensively described using molecular, biochemical, anatomical and physiological levels of analysis; less empirical work has related these functional changes to the behavior of the aging organism. Among the numerous behavioral responses affected by aging are changes in baseline pain thresholds and analgesic responsivity. Given the recent discoveries that increases in pain thresholds occur following acute exposure to physiological stressors in young adult rats (see review: Bodnar, 1984), the question arises as to whether aging alters analgesic stress responses in a similar manner to aging's overall effects upon adaptation. In this regard, analgesic and other adaptive responses to stress and to opiates are mediated through adreno-cortical, sympatho-medullary, and neurohypophyseal neurotransmitter systems (Bodnar, 1984; Axelrod and Reisine, 1984; Basbaum and Fields, 1984), which are compromised during the aging process. Although the general reactivity of the aged organism has been characterized by a reduction in anabolic activity and an increase in catabolic activity (McGeer, 1971), more specific alterations have been described such as reductions in levels and receptor binding of glucocorticoids (Roth, 1974; Tang and Phillips, 1978) , opioids (Dupont, 1981; Gambert, 1980, 1981; Messing, 1980) and catecholamine neurotransmitters (Finch, 1973; Estes and Simpkins, 1980). Moreover, age changes in the functional

activity of these systems are especially accentuated following exposure to acute and chronic stress.

Given the integral role that these response systems play in mediating stress and morphine analgesia in the young adult rodent, the purpose of the present experiment was to assess systematically, across age groups, the analgesic responses of the rat following morphine administration and following acute exposure to two physiological stressors, 2-deoxy-D-glucose glucoprivation and cold water swims. Further, multiple pain measures and the assessment of other physiological responses following stress or morphine exposure were used to clarify the extent and specificity of adaptive changes in rats of various ages. The following sections briefly review a) endogenous pain inhibitory systems that are activated by morphine and/or physiological stressors in young adult rodents; b) the effects of aging upon endocrine and neural systems; and c) the effects of aging upon baseline pain thresholds, morphine analgesia and stress responses.

A. Endogenous Pain Inhibitory Systems:

Endogenous opioid and nonopioid pain inhibitory systems have been shown to modulate the analgesic states observed following the administration of opioids or acute external stressors in young adult rodents. Characterization of the opioid pathway subserving pain inhibition was fostered by the discovery of endogenous ligands (i.e., beta-endorphin and the enkaphalins) which have been shown to bind to opiate receptors in the dorsal horn of the spinal cord, the medullary nucleus raphe magnus, and the midbrain periaqueductal gray (Hughes, et al., 1975; Goldstein, 1976; Pert and Snyder, 1973; Kuhar, Pert and Snyder, 1973). Further, both opiates and opioid peptides produce increases in pain thresholds following microinjections into these discrete anatomical structures (Belluzi et al., 1976; Pert, Pert, Chang and Fong, 1976; Jacquet et al, 1976; Yaksh and Rudy, 1978; Yaksh, 1981). This descending system is thought to involve direct excitatory projections from the periaqueductal gray (PAG) to such rostral ventral medullary structures as the nucleus raphe magnus, the nucleus reticularis magnocellularis and the nucleus reticularis paragigantocellularis lateralis (Basbaum and Fields, 1984; Fields and Basbaum, 1978). These nuclei, in turn, project through the dorsolateral funiculus of the spinal cord to dorsal horn laminae where they produce profound physiological inhibition upon nociceptive neurons. This descending inhibitory system has been postulated to mediate the analgesia observed following direct electrical stimulation (stimulation produced analgesia (SPA)) of either the PAG (Mayer,

Wolfe, Akil, Carder, and Liebskind, 1971), the nucleus raphe magnus (Proudfit and Anderson, 1975), and the nucleus gigantocellularis (Yaksh and Rudy,1978; Messing et al., 1977). A common substrate of pain inhibition for morphine analgesia and for SPA has been proposed (see review: Mayer and Price, 1976) based upon observations of synergy (Samanin and Valzelli, 1971), partial cross tolerance (Mayer and Hayes, 1975), reversal by the opiate receptor antagonist nalaxone (Oliveras, Hosobuchi, Redjemi, Guilbaud, and Besson, 1977; Akil, Mayer, and Liebeskind, 1976, but see Pert and Walter, 1976; Yaksh,Yeung and Rudy, 1976), and loss of each effect following lesions placed in either the raphe magnus or the dorsolateral funiculus (Basbaum, Marley, O'Keefe and Clanton,1977; Rhodes, 1979; Dostrovsky and Deakin, 1977). Similarly, high concentrations of opioid peptides and the opiate receptors are found in areas that elicit morphine analgesia and SPA (Watson et al, 1979; Mayer and Price, 1976). Moreover, both morphine and electrical stimulation directly applied to the PAG excite cells in the nucleus raphe magnus and inhibit nociceptors in the dorsal horn of the spinal cord (Oliveras et al., 1977; Fields and Basbaum, 1978). In general, while morphine analgesia appears to be primarily mediated by either spinal or supra-spinal opioid systems (e.g., Yaksh and Rudy, 1978), the mediation of SPA appears to be under both opioid and nonopioid control. For example, analgesia elicited following stimulation of the ventral PAG, but not the dorsal PAG, is reversed by nalaxone (Cannon, Prieto, Lee, and Liebeskind, 1982), and excites cells in the raphe magnus that eventually inhibit cells in the dorsal spinal column (Fields and

Basbaum, 1978; Fields and Anderson, 1978; Oliveras et al., 1974).

Another means by which endogenous mechanisms subserving pain inhibition are activated is following acute exposure to such experimental manipulations as immobilization, centrifugal rotation, hypertonic saline injections, food deprivation, inescapable footshock, 2-deoxy-D-glucose (2DG) and insulin glucoprivation, and cold water swims (CWS) (Akil, Madden, Patrick and Barchas, 1976, Hayes, Bennett, Newlon and Mayer, 1978;1976; Bodnar, Kelly, Brutus, Mansour and Glusman,1978; Amir and Amit, 1978; Bodnar, Kelly, Spiaggia and Glusman, 1978). All of these manipulations are considered stressors in that they release glucocorticoids from the adrenal cortex, adrenocorticotrophic hormone (ACTH) from the anterior lobe of the pituitary gland, and catecholamines from the adrenal medulla and central nervous system (Axelrod and Reisine, 1984). The elicitation of analgesic responses by a variety of environmental stimuli otherwise differing in their physical properties has led to the suggestion that there is a common basis underlying these analgesic responses. As the mechanisms mediating the analgesic effects of these stimuli share both similarities and difference with morphine induced analgesia (see: Watkins and Mayer, 1982; Terman et al., 1984; Bodnar, 1984), it appears that multiple physiological substrates modulate different forms of pain. The following section will focus on two analgesic stressors, 2DG and CWS, since they produce different profiles of analgesic responses, are amenable to the measurment of other physiological variables, and are the variables under study in the present experiments.

2DG Analgesia

2DG, a glucose analogue, selectively crosses cell membrames and inteferes with normal cellular metabolism (Wick, Drury, Nakada and Wolfe, 1957). Following administration of 2DG, many stress related physiological responses occur, including glucoprivation, peripheral sympatho-medullary and pituitary-adrenal discharge, hyperglycemia and alterations in cental catecholaminergic functioning (Wick et al., 1957; Brown, 1962; Smith and Epstein, 1969; Smith and Root, 1969; Himsworth, 1970). Moroever, acute administration of 2DG produces analgesia (Bodnar et al., 1978; Bodnar et al., 1981) and hyperphagia (Smith and Root, 1969; Smith and Epstein, 1966; Berthoud and Mogenson, 1977; Engeset and Ritter, 1980) following both systemic and central injections. The analgesic response to 2DG appears to be mediated by both opioid and nonopioid pain inhibitory mechanisms. Evidence for opioid involvement has come from the observations that 2DG induced analgesia displays tolerance following repeated injections (Bodnar, Kellly, Brutus and Glusman, 1978; Bodnar et al., 1983), and partial cross tolerance with morphine (Spiaggia, Bodnar, Kelly and Glusman, 1979; Bodnar, Kramer, Simone, Kirchgessner and Scalisi,1983), is potentiated in hypophysectomized rats and attenuated in animals with lesions placed in the PAG (Bodnar,Kelly and Glusman, 1979, Spiaggia et al, 1979; Brutus et al., 1979). However, 2DG and morphine analgesia also display dissimilar effects. 2DG analgesia is not altered by the opiate antagonist, nalaxone (Bodnar, Kelly

and Glusman, 1979), exhibits full and reciprocal cross tolerance with the nonopioid mediated CWS analgesia (Spiaggia, Bodnar, Kelly and Glusman, 1979), and displays additive rather than multiplicative synergy when subanalgesic doses of morphine and 2DG are applied (Spiaggia et al., 1979).

Central and peripheral nervous system mechanisms have been proposed for 2DG hyperphagia as well (Smith and Epstein, 1969; Ritter and Neville,1976). Because the time courses for cellular glucoprivation and hyperphagia differ in animals deprived of food or infused with glucose after 2DG administration (Ritter,Roelke, and Neville,1978; Ritter, Bellin and Pelzer, 1981), it is unlikely that 2DG hyperphagia is merely an epiphenomenon of cellular glucoprivation. On the other hand, support for the central modulation of 2DG hyperphagia derives from the observations of increased activity of hypothalamo- pituitary-adrenal and sympatho-medullary axes, and from the finding that systemic 2DG injections prevent both norepinephrine depletive and hyperphagic responses induced by acute foot shock (Ritter, Pelzer and Ritter, 1978; Ritter and Ritter, 1977). Additional clarification regarding the mediation of 2DG effects has been obtained by comparing the underlying mechanisms of 2DG analgesia and hyperphagia, each of which is elicited following both intra-cerebroventricular and systemic administration (Smith and Epstein, 1969; Engeset and Ritter, 1980; Bodnar, Kelly, Brutus, Mansour, and Glusman, 1978; Bodnar Merrigan and Wallace, 1981). These studies have suggested a dissociation between the two responses. It has been argued that 2DG hyperphagia, but not 2DG analgesia, is reduced by nalaxone and

lateral hypothalamic lesions (Lowy, Maickel and Yim, 1980; Grossman and Grossman, 1973; Bodnar, Kelly and Glusman, 1978; Bodnar et al, 1983). On the other hand, 2DG analgesia, but not 2DG hyperphagia, is potentiated by dopamine receptor antagonists and reduced by dopamine receptor stimulants (Bodnar and Nicotera, 1982; Stricker and Zigmond, 1974; Berthoud and Mogenson, 1977). Moreover, 2DG analgesia, but not 2DG hyperphagia, shows tolerance to repeated injections (Bodnar, Kelly, Brutus and Glusman, 1978). In summary, although central mechanisms mediate both 2DG induced hyperphagia and analgesia, they appear to be dissociated phenomena; both, however, are influenced by opioid and nonopioid manipulations.

CWS Analgesia:

Acute exposure to CWS produces both analgesic and hypothermic responses in young adult rodents (Bodnar, Kelly and Glusman, 1978). Although both responses are potentiated in rats pretreated with the alpha-noradrenergic receptor agonist, clonidine (Bodnar, Merrigan and Sperber, 1983), and are reduced in rats pretreated neonatally with the circumventricular neurotoxin, monosodium glutamate (Badillo-Martinez et al.,1982), dissociations between these two responses are apparent. CWS analgesia, but not CWS hypothermia shows adaptation after repeated exposure (Bodnar, Kelly, Spiaggia, Glusman, 1978), and is reduced in hypophysectomized animals (Bodnar, Glusman, Brutus, Spiaggia, Kelly,1979), and in animals treated with D-phenylalanine (Bodnar, Lattner, Wallace, 1980). These findings suggest that whereas the analgesic effect reflects the stressful consequences of the swims, the hypothermia reflects an adaptive thermal and vasoconstrictive

response to direct exposure to cold.

CWS analgesia appears to possess both opioid and nonopioid properties (Bodnar, 1984). Morphine and CWS analgesia are both potentiated in adrenalectomized rodents (Holoday et al., 1979; Glusman, Bodnar, Mansour and Kelly, 1980) and in rodents pretreated with neuroleptics (Bodnar and Nicotera, 1982). In addition, both morphine analgesia and CWS analgesia are reduced following neonatal monosodium glutamate administration, and following tail pinch stress (Simone and Bodnar, 1982; Bodnar, Abrams, Zimmerman, Krieger, Nicholson and Kizer, 1980; Badillo-Martinez et al., 1984). Moreover, CWS analgesia has been associated with changes in endogenous opioid peptide content (Pert and Bowie, 1979). However, while nalaxone completely eliminates morphine analgesia (see: Martin, 1967; Sawynok et al., 1979), variable effects have been reported for CWS analgesia. The effectiveness of naloxone to inhibit CWS analgesia apears to be dependent upon such variables as the water temperature, swim duration, post-swim interval, swim parameter, and the magnitude of analgesic response induced in individual animals (Bodnar, Kelly, Mansour, and Glusman,1979; Bodnar and Sikorszky, 1983; Giradot and Holloway, 1984).

The suggestion that CWS analgesia is also mediated by a non-opioid pain inhibitory system is supported by the lack of cross tolerance between CWS and morphine analgesia (Bodnar, Kelly, Steiner and Glusman, 1978). Moreover, naloxazone, a high-affinity opiate antagonist (Pasternak et al., 1980), reduces morphine analgesia while potentiating CWS analgesia (Kirchgessner et al., 1982). Further, D-phenylalanine, a putative anti-enkephilinase

(Ehrenpreis, Balagot, Comaty and Myles, 1979), reduces CWS analgesia (Bodnar, Lattner, and Wallace, 1980), and potentiates morphine analgesia (Ehrenpreis et al., 1979). A variety of other manipulations result in a dissociation between the two analgesic responses. Hypophysectomy potentiates morphine analgesia (Bodnar, Glusman, Brutus, Spiaggia and Kelly, 1979) while reducing CWS analgesia, but in Brattleboro rats with vasopressin deficiencies, only CWS analgesia, but not morphine analgesia is reduced (Bodnar, Zimmerman et al., 1980). On the other hand, morphine analgesia, but not CWS analgesia, is reduced by depletion of serotonin by lesions placed in the serotonergic midbrain raphe nuclei (Tenen, 1968; Samanin, Ghezzi, Mauron, Valzelli,1973; Bodnar, Kordower, Wallace and Tamir, 1981).

In summary, in the young adult rodent, the analgesia induced by exposure to acute physiological stressors as CWS and 2DG, is modulated by both opioid and nonopioid mechanisms. This is suggested by the fact that stress induced analgesia and associated physiological responses share common and distinctive properties with the analgesia seen in response to morphine administration, and are both similarly and differentially affected by neuroendocrine and neurotransmitter manipulations. The following section examines the status of the endogenous opioid, endocrine and neural systems in the aging rodent.

B. Effects of Aging on Endocrine and Neural Systems:

The empirical literature on the effects of aging on endogenous opioid levels and activity in various regions of the brain with regard to both endorphins and enkephalins is reviewed here. Also reviewed are age differences in the levels and reactivity of both peripheral and central endocrine response systems with regard to glucose tolerance, thyroid functioning, and the hypothalamo-pituitary-adrenal axis. Finally to be reviewed is the empirical literature on the concentration, activity, and receptor binding, of the neurotransmitters dopamine, norepinephrine, serotonin, GABA, substance P, and acetylcholine as a function of age in rodents.

Aging and Opioid Levels:

The endogenous opioid peptide, beta-endorphin, which has been shown to have thermoregulatory, analgesic, and anxiolytic effects (Kastin, Jemison and Coy, 1979; Bolles and Fanselow, 1982), is generally reduced in the aged rodent's brain. Reductions in the beta-endorphin concentration in old (20-24 mo) as compared to young (3-6 mo) rats have been observed in the hypothalamus and striatum, but not in the pituitary gland, the median eminence or the frontal cortex (Barden et al., 1981; Gambert, Garhwaite, Pontzer and Hagen, 1980). Similarly, opiate receptor binding studies reveal a progressive reduction in the receptor concentration, without changes in affinity in the frontal cortex, striatum, amygdala and hippocampus of older (24 mo) as compared to younger (2-3, 6-12 mo) rats (Hess, Joseph and Roth, 1980). In contrast, immunoreactive beta-endorphin content levels are equivalently elevated in the

plasma across ages (3-5 vs 19-23 mo) in the hypothalamus (Forman, Sonntag, Van Vugt and Meites, 1981). Both hormonal and neurotransmitter systems have been implicated in modulating these age related reductions. A parallel developmental profile for immunocreactive ACTH levels has been observed in the striatum, but not in the hypothalamus or pituitary (Gambert et al., 1980). Further, whereas senescent (20-24 mo) rats made hyperthyroid by injections of T3 demonstrated an elevation in beta-endorphin levels, young (6 mo) hypothyroid rats displayed reduced beta-endorphin levels, equivalent to the basal levels observed in older rats (Gambert, 1981). As the reduction in beta-endorphin levels is also accompanied by elevated prolactin levels, less efficient tubero-infindibular dopaminergic and opiate system interactions have also been implicated (Barden et al., 1981).

The enkephalins have been shown to mediate pain and emotional responsivity and to interact with hormonal and neurotransmitter systems (Dupont et al.,1977; Bolles and Fanselow, 1982). Comparison of the concentrations of met and leu-enkephalins in discrete nuclei of the senescent (24-26mo) and mature (4-5mo) rat brain has revealed significant reductions in met-enkephalin with increase in age in the suprachiasmatic, arcuate, and premamillary nuclei. Leu-enkephalin concentrations were reduced in the medial preoptic, suprachiasmatic, paraventricular, ventromedial, and premamillary nuclei (Dupont, et al., 1981). In contrast, an analysis of met-enkephalin levels in senescent female rats (22-24mo) revealed equivalent hypothalamic levels when compared to younger female rats (4-6mo), but a significantly higher met-enkephalin concentration in the anterior

lobe of the pituitary gland (Kumar, Chen and Huang 1980). An interactive role with gonadal reproductive systems was postulated, since older pseudopregnant rats had the highest concentrations, followed by those with irregular cycles. Older rats exhibiting constant estrus showed the lowest enkephalin concentrations.

Receptor binding studies have described a reduction in the density of binding sites for 3H-dihydromorphine in the thalamus, midbrain and cortex of older female rats (20 mo) with equivalent levels being observed in the striatum when compared to younger rats (4 mo) (Messing et al.,1980). Moreover, the older group failed to demonstrate high and low affinity binding sites, and instead receptor affinity was characterized by a steady intermediate level. In contrast, reduced opiate receptor density and higher opiate binding affinity was observed in the frontal poles of the aged (26 mo) male rat (Jensen, Messing, Martinez, Jr., Vasquez, and McGaugh, 1980).

Thus, a review of the empirical literature suggests that aging is accompanied by reduced concentrations and reduced receptor binding of the opioid peptides beta-endorphin and met and leu-enkephalin. However, since there is a lack of consistency across studies with regard to the gender and ages of rodents used, the exact nature and locus of age-related deficits are difficult to identify. Moreover, age-related comparisons are hindered by the fact that young-versus-old rather than life span developmental comparisons are made. In addition, although most investigators concur in suggesting that reductions in opioid levels and binding may be accompanied by less efficient neurotransmitter and endocrine

system functioning, the exact nature of this interaction remains to be clarified.

Aging and Endocrine Response Systems

Alterations in endocrine functioning with increasing age have been suggested by the findings of less efficient ability of endocrine glands to maintain hormonal blood levels, to activate and bind to target tissues, and by the differential responsivenss of the hypothalamic-pituitary axis to feedback regulation by hormones and neurotransmitters. Progressive glucose intolerance occurs with aging across mammalian species (O'Sullivan, Mahan, Freedlender and Williams, 1971; DeFronzo, 1981, Brachero-Romero and Reaven, 1977) and has been attributed to reduced insulin responsivity by pancreatic islets (Kitahara, et al., 1979), impaired glucose metabolism (Reaven et al., 1979), and the elevated sensitivity of hypothalamic receptors to inhibition (Dilman et al, 1979). Thyroid hormone functioning appears to be compromised by aging as well, with reduced levels of both thyroxine (T4) and Triodothyronine (T3) (Klug and Adelman, 1979; Sartin, Pritchett and Marple, 1977) and less sensitivity to thyroid hormone (Denckla, 1974) being reported. The reduced levels of thyroid hormone in older rodents has been related to reductions in beta-endorphin, which is reversed in hyperthyroid senescent rats (Gambert et al., 1981).

Age-dependent alterations in hypothalamo-pituitary interactions have been observed in both the anterior and posterior lobes of rodents. For instance, although lower plasma lutenizing hormone (LH) and follicle stimulating hormone (FSH) levels have been observed in the older (22 mo) male rat (Shaar, Euker, Riegle, and

Meites, 1971), aged (17-26 mo) females do not show cyclic fluctuations in serum LH and FSH levels and their pituitary glands are less responsive to LHRH (Riegle and Miller, 1978; Peng and Huang, 1973). Reduced androgen levels, reduced estradiol receptors and elevated prolactin levels have also been reported in aged (14-27 mo) rodents (Choukayiska and Bassilevea-Popova, 1977; Kanung, Patnaik and Koul, 1975; Shaar et al, 1971, Bruni, Huang, Marshall, and Meites, 1977). Age related changes in posterior pituitary activity have been reported, with reduced levels of vasopressin in the hypothalamus (Zbuzek and Wu, 1982), the posterior lobes (Rodeck, Ledris and Heller, 1960) and the plasma and urine (Turkington and Everitt, 1976) of sensescent rodents. These changes have been associated with functional impairments in: dehydration induced responses (Sladek,McNeill, Gregg, Blair and Baggs, 1981), learning and memory abilities (Cooper, McNamara and Thompson, 1980), and reduced immunostaining of magnocellular neurons (Watkins and Choy, 1980). In contrast, elevated plasma A-vasopressin levels, associated with increased paraventricular neurosecretory activity, were seen in old (32 mo) as compared to young (3 mo) rats (Fliers and Swaab, 1983). Changes in adrenocortical responses as a function of age have been suggested as contributing to impaired homeostatic functioning and responsivity to stress (Dilman, 1971; Tang and Phillips, 1978). These have primarily been attributed to the reduced sensitivity and/or elevated threshold of the hypothalamic-pituitary axis to feedback inhibition by glucocorticoids (Dilman, Ostroumova, Tsyrlina, 1979). Glucocorticoid receptor binding has been reported to show a

progressive reduction in the cortex of rats from 3-25 months of age (Roth, 1974,1976). However, while basal levels of ACTH have been reported to show an increase (Tang and Phillips, 1978), a decrease (Gambert et al, 1980), or no changes with age (Finch, 1969; Hess and Riegle, 1972), there is an impaired ability of older rodents to increase ACTH levels when exposed to stress (Tang and Phillips, 1978, Gambert et al, 1980; Britton, Rotenberg and Adelman, 1975).

In summary, although some endocrine systems show clear reductions in levels and turnover with increasing age (thyroid hormones, gonadotrophic hormones, glucose/insulin hormones), there is controversy regarding the baseline status of others (ACTH and vasopressin). In addition, the nature of the interaction between these age- related changes and other neural and endocrine systems, as well as their role in adaptation to stress, is not yet well understood. Finally, the level at which age-related deficits occur, i.e., target tissue binding, feedback regulation, or inefficient release and/or maintenance regulation, remains to be clarified.

Aging and Neurotransmitter Systems

Catecholamines: Both the levels and turnover rates of catecholamine neurotransmitters, as well as their synthesizing and degredative enzymes, have been found to be altered in the aged rodent. These changes have been related to the impaired modulation of both behavioral and neurohumoral activites. Although whole brain levels of catecholamines in mice have not been found to differ as a function of age (Finch, 1973), aged (24 mo) rats showed lower dopamine (DA) levels in the neostriatum, median eminence and posterior pituitary, reduced DA reuptake in the hypothalamus and striatum, and a significant slowing of turnover rates for both DA and norepinephrine(NE) in the brainstem and hypothalamus than younger (8-12 mo) rats (Jonec and Finch, 1975; Finch, 1973). With increase in age, a general reduction in the levels of all monoamines in the cerebral cortex of female rats has been reported, though the time course of the changes was specific to the individual transmitters (Timiras et al.,1980). DA showed a gradual reduction with age (6-22mo) but NE levels showed a marked reduction until 11 mo of age and then stabilized. Whereas DA is only reduced in the striatum, lower NE levels and turnover rates have been reported in the hypothalamus (Miller, 1976), and brainstem (Sun, 1976; Ponzio,1978; Estes and Simpkins, 1980) of older rats. The synthesizing enzyme tyrosine hydroxylase is reduced in the caudate and (McGeer, 1971) olfactory tubercle (Reis, Ross, and Joh, 1977), but the degredative enzyme, catechol-O-methyl transferase, is elevated in older rats (Stratmentinoli, Gualano,Catto and Algeri,

1977; Samorajski and Rolstein, 1973).

Gradual reductions in number, but not affinity, of receptor binding sites have been reported for DA in the striatum (Govoni,Loddo, Spano and Trabucchi, 1977), substantia nigra, hypothalamus, frontal cortex, and anterior limbic cortex of aged rats (Govoni,Memor, Saiani, Spano and Trabucchi, 1980; Makman et al., 1980). The age related changes in receptor binding seem to be most affected by the adenyl cylcase mediated systems (D1 receptors) rather than those mediated by D2 receptors (Burchinsky, 1984). Reduced beta-adrenergic receptor binding has been noted in the cerebral cortex (Misra, 1980), the brainstem and cerebellum (Maggi, 1979), as well as in the striatum, cerebellum and pineal gland (Greenberg, 1978) of aged rats. Both reductions and equivalent number of cortical binding sites have been reported for alpha adrenergic receptors in the cortex (Misra, 1980; DeBlasi, Cotecchia, Mennini, 1982). Norepinephrine-stimulated CAMP activity has been shown to be reduced in the senescent rat's cerebellum (Schmidt and Thornberry, 1978) and cortex (Berg and Zimmerman, 1975).

Serotonin: The results of majority of studies concur that serotonin does not seem to be markedly affected by aging in mice and rats (Finch, 1973; Simpkins, Mueller, Huang, and Meites, 1977). However, reductions in serotonin levels in specific brain regions have been observed in the raphe nuclei and hippocampus of old (24 mo) as compared to young (1 mo)rats, paralleling a reduction in the synthesizing enzyme tryptophan hydroxylase (Meek, Bertilsson, Cheney, Zsilla and Costa,1977). Hypothalamic turnover in the senescent (21 mo) rat was found to increase in response to the

monoamine oxidase inhibitor, pargyline, and was paralled by higher serotonin metabolite levels than that seen in younger (3-4 mo) animals (Simpkins, 1977). Yet serotonin receptor binding studies reveal no age differences in the striatum and hypothalamus of senescent mice and rats (Jonec and Finch, 1975; DeBlasi et al., 1982).

Gamma Amino Butyric Acid (GABA): No significant age changes for GABA (Fonda,Acree and Auerbach, 1973) or its metabolite have been observed in the brainstem and cerebellum of older (26 mo) rats (Epstein and Barrows,1969), although a small reduction has been observed for the metabolite in the caudate (McGeer, Fibiger, McGeer, and Wickson, 1971). GABA receptors are equally dense in young and old rodents in the cerebellum, cortex, and striatum (Maggi, Schmidt, Gnetti and Enna, 1979), with small reductions being reported for the substantia nigra and hypothalamus of older (24-30 mo)as compared to younger (3-4 mo) animals (Govoni et al., 1980).

Substance P:

Although substance P is involved in pain transmission (Frederickson et al., 1980; Wallace et al., 1980), there is little research on its status in the aged rodent. Brain estimates of substance P in aged (28 mo) rodents have revealed reductions in the hypothalamus, but not in the frontal cortex, thalamus, caudate, globus pallidus and substantia nigra as compared to younger (4, 11 mo) rodents (Buck, Deshmulch, Burks and Yamamura, 1981). A reduction in hypothalamic substance P was observed in the anterior and ventromedial nucleus in older rodents (Dupont, Savard, Merand, Labrie, and Boissier, 1981).

Acetylcholine: The cholinergic synthesizing enzyme, choline acetyl transferase, has been reported to be reduced in the caudate and cerebellum (Meek et al, 1977; McGeer et al., 1971), although no reductions have been observed in the nucleus accumbens, the interpendicular nucleus, the locus coerulus or the septum of old (24 mo) as compared to young (1 mo) rats (Meek et al., 1977). In contrast, the status of choline acetyl transferase in the hippocampus of older rats is controversial, with both reduced levels and no differences being reported (Vijayan, 1977; Meek et al.,1977). Reduced levels of the degredative enzyme, acetylcholinesterase, have been found in whole brain as well as the hippocampus, striatum and cerebellum of senescent rodents (Reis et al., 1977; Moudgil and Kanungo,1973). Cholinergic receptor binding is reduced in number but not affinity in the whole brain, striatum, cerebellum and cortex of old (18-26 mo) as compared to young (3-5 mo) rats (Freund,1980; Jensen, 1978; Morin and Wasterlain, 1980).

In summary, the developmental profile of neural and endocrine systems outlined in the preceding section suggests that these systems are differentially affected by the aging process. Whereas clear reductions have been observed in specific brain regions for the activity, concentration and receptor binding levels of the endogenous opioids, catecholaminergic, and cholinergic neurotransmitter systems with increases in age, other systems, e.g., GABA, substance P, and serotonin, are less affected by increase in age. In the endocrine system, impaired functional reactivity has been found in older rodents, as reflected in both the glucose/ insulin and hypothalamo-pituitary-adrenal axes. The following section

examines whether corollary changes are observed on a behavioral level, by examining age-related responses to pain inducing stimuli, morphine administration, and physiological/environmental stressors.

C. Effects of Aging on Pain Thresholds, Morphine Analgesia and Stress Responses:

Aging and Basal Pain Thresholds: The reports of the effects of aging on basal pain thresholds are inconsistent, with some indicating increased thresholds (Pare,1969; Nicak, 1971; Hess, Joseph and Roth, 1980), others decreased thresholds (Gordon, Scobie and Frankel,1978; Kavaliers, Hirst and Teskey, 1983; Chan and Lai, 1982), and others no threshold changes (Lippa, 1980, Saunders, Paolino, Bousquet and Miya, 1974; Pare, 1969; Webster, Shuster and Eleftheriou,1976; Spratto and Dorio, 1978). These conflicting findings may be related to the use of different pain inducing stimuli (e.g., thermal, electrical, mechanical), different response measures (vocalization, orientation, jump, tail-flick and escape), species, weight, and the lack of consistency in the definition of "young" and "old" groups in the various experiments. For example, older male rats (16 mo) displayed higher aversive thresholds for grid shock on a spatial preference task than either cohort-matched females or younger rats (34, 80, 220 days) of both sexes (Pare, 1969). The increased thresholds of the older males as compared to older females were attributed to their greater body weight. In contrast, reductions in shock thresholds were found as a function of age (3 mo > 12 mo > 22 mo) for both sexes when flinch or orienting responses (gross movements)

were the response measures (Gordon, Scobie, and Frankel, 1978). Other studies found no evidence of age changes in electrical shock thresholds on the jump and vocalization tests (Lippa, 1980; Saunders, et al., 1974), nor elevated pain thresholds to either electrical or mechanical stimuli (Nicak, 1971).

Similarly, the empirical findings regarding age changes in baseline reactivity to a heat stimulus are not unequivocal. Although older (24 mo) and younger (2-3 mo) mice display similar tail flick latencies (Webster, et al.,1976), the older (20-30 mo) mice have shorter response latencies on a hot plate test (Kavaliers, et al, 1983). Also, older (10 mo) and younger (2 and 6 mo) rats display similar tail flick latencies to a heat stimulus (Spratto and Dorio, 1978), whereas higher tail flick latencies are observed when their tail is immersed in 55C water (Hess, Joseph and Roth, 1980). Further, hot plate latencies have been found to both decrease (Chan and Lai, 1982) and increase (Hess et al., 1980) with increase in age. Aging and Morphine Responses: Whereas morphine analgesia increases with increasing doses in young adult rodents (Johannesson and Becker,1973), this relationship is less clear in mature and older rodents. This lack of clarity is attributed at least in part, to in consistencies in the choice of pain measures, and the use of other morphine-related behaviors across experiments. For example, progressive reductions in morphine (10mg/kg) and clonidine analgesia as a function of age (2, 5, 12 mo) on the hot plate test were found to be related to reduced concentrations of dopamine, acetylcholine, and opiate receptors (Chan and Lai, 1982). Similar reductions in morphine (5mg/kg) analgesia were observed in mice across ages (2-30 mo) on the tail flick and hot plate

tests (Webster, Shuster and Eleftheriou, 1976; Kavaliers et al., 1983); these effects were attributed to morphine's slower absorption and distribution and lower plasma melatonin levels, respectively, in the aged as compared to the young mouse. Finally, dose-response studies have shown that younger rats (1.5 mo) displayed greater analgesia to a 5 mg dose of morphine than older rats (10 mo), but the converse was true for a dose of 7.5 mg/kg of morphine (Spratto and Dorio, 1978). Moreover, the administration of a high dose of morphine (30mg/kg) resulted in higher concentrations of morphine in both the plasma and brains of the older rat (10 mo) over a 4 hour period.

Other physiological behaviors in response to morphine or opioid acting drugs have been found to be related to aging. Older rats (20 mo) display an increased hyperthermic response to low doses of morphine (5mg/kg) and a reduced hypothermic effect to high doses of morphine (25mg/kg) compared to younger (3-5, 10-12 mo) rats (McDougal, Marques, and Burks, 1980). This was not related to an inability of the older animals to thermoregulate, since ethanol administration produced thermoregulation. Older rats are less sensitive to morphine's respiratory depressant effects (Spratto and Dorio, 1978), show a reduced suppression of feeding behavior following the opiate antagonist, nalaxone (Gosnell, Levine, and Morley, 1983), and a smaller increase in food intake following the opiate agonist, butorphenol (Gosnell et al., 1983).

In summary, a review of the empirical literature with regard to baseline pain reactivity suggests that many of the reported age-related differences are inconsistent. These differences may be attributed to the lack of constancy in pain inducing stimuli, pain response measures

and age groups used. Moreover, although most investigators concur in describing a reduction in morphine analgesia with increase in age, others disagree. These inconsistent reports may be attributed to the lack of control of age-related baseline pain responses, as well as to differences in age, drug dose and post-injection time interval at which pain responsivity was studied.

Age and Stress Responses: The altered functioning of neurosecretory processes associated with the hypothalamic-pituitary-adrenal axis of aging rats appears to disrupt the mechanisms necessary for adaptive responses to prolonged stress (Frolkis, Bezrukov, Duplenko and Genis, 1972; Timiras, 1978). The attempt to understand physiologically adaptive behaviors as a function of aging has been facilitated by a comparative analysis of stress responses in the young, mature and senescent rodents after exposure to cold (4-10C), with most studies reporting a greater reduction and duration in core body temperatures with increase in age. The older rodent's reduced ability to thermoregulate has been associated with reduced levels of either homovanillic acid in the striatum or tyrosine hydroxylase in the hypothalamus, increased levels of 5-hydroxyindole-acetic acid, and a delay in the induction of liver tyrosine amino transferase (Brady, Brown and Thurmond, 1982; Thurmond and Heishman, 1984; McDougal, Marques, Burks, 1981; Algeri et al, 1982). Similar effects have been observed following acute cold stress by submersion in cold water solutions, with less efficient thermoregulatory control in older rats manifested by greater reductions in core body temperature and longer recovery times (Segall and Timiras, 1975; Thurmond and Heishman, 1984). For instance, 25 month old rats exposed to a 1 minute submersion in a

4C water bath displayed equivalent hypothermia to younger (10 mo) rats exposed to the same bath temperature for 12 minutes. Moreover, these older rats continued to show greater declines in body temperature after removal from the water and took longer to recover than did the younger animals (Hamm, 1981). These age differences are accompanied by a greater reduction in motor activity, and a smaller increase in corticosterone levels, but no differences in cold induced amnesia in old as compared to young rodents.

Both central and peripheral factors have been implicated in the differential responsiveness of older rodents to the stress induced by exposure to a cold environment or swim. The findings of impaired gluconeogenesis and of a delay in the induction of liver tyrosine amino hydroxylase have implicated the liver and adrenal cortex (Finch, Foster and Mirsky, 1969; McDougal et al., 1981). On the other hand, other investigators suggest alterations in central neurotransmitter functioning. This suggestion is supported by the finding that senescent rodents showed a greater reduction in norepinephreine and serotonin levels, impaired adrenocortical and catecholaminergic system responsiveness, and an imbalance between DA and 5HT systems following cold water swims (Brady, Brown and Thurmond, 1980; Thurmond and Heishman,1984; Algeri et al.,1972; Ritter and Pelzer, 1978) compared to younger rodents.

Impaired regulatory homoeostatic and adaptive functioning are also seen in aging rodents in response to physiological challenges. Delays in the induction of hepatic glucokinase activity in response to a glucose challenge were found to be related to the reduced availability of glucocorticoids in older (24 mo) as compared to young adult and

mature (2,12 mo) rats (Sartin et al., 1980). A reduced ability of older rats to increase corticosterone secretion has also been evidenced in responses to short-term starvation and after challenge with maximal ACTH doses (Britton,Rotenberg and Adelman, 1975; Hess and Riegle, 1970).

The differential effects of stress on old (24-27mo) as compared to young adult (3-6mo) rats have been evaluated following exposure to either a novel environment or ether vapors. Older rats were less able to increase corticosterone levels, an effect which appeared to be degree-dependent since no age differences in corticosterone were observed following mild stress (Brett, Chung, Coyle and Levine, 1983; Hess and Riegle, 1970). Similar increases in ACTH secretion occurred following short term stress (2.5 min of ether) in young (2.5 mo) and old (26 mo) rats; however, older rats fail to increase further ACTH secretion following a 15-min exposure to ether (Tang and Phillips, 1978).

Another stressor, electric foot shock, elicits less sympatho-adrenal medullary discharge of norepinephrine and epinephrine in aged (24mo) than in younger rats (4mo) after a 1 minute exposure (McCarty, 1981). Central catecholaminergic systems are also affected in that aging rats exposed to footshock display reduced brainstem dopamine levels (Welsh and Gold, 1984), reduced hypothalamic tyrosine hydroxylase levels (Algeri, Calderini, Lomuscio, Toffano, Ponzio, 1982), and a smaller acute reduction in norepinephrine forebrain and brainstem levels. In contrast, chronic exposure to footshock stress appears to result in a greater depletion of norepinephrine levels which is accompanied by a longer recovery period in the older than in the

younger rat and mouse (Ritter and Pelzer, 1978; Samorajaski, Rolsten, and Ordy, 1971). Exposure of older (28 mo) rodents to short-term (3min) immobilization stress is accompanied by greater reductions in blood pressure and heart rate (Chuieh, Nespor and Rapoport, 1980) compared to younger (3, 12 mo)rodents. Moreover, exposure of aged rats to long term immobilization stress (30 minutes-3hours) resulted in a delay in the maximal peak of norepinephrine release, a reduction in beta adrenergic responses, elevated plasma epinephrine levels, and prolonged activation of NE neurons in the hypothalamus, amygdala, and pons-medulla (Chuieh et al., 1980; Ida et al., 1982; Ida et al., 1984).

In summary, examination of the developmental course of adaptive and coping responses to pain inducing stimuli, to morphine and glucose challenges, and after exposure to ether, footshock or immobilization stress, indicates differential responsivity on the part of the aged rodent in comparison with its younger counterpart. These changes with age have been associated with impaired peripheral and central endocrine, enzymatic, glucocorticoid, and catecholimanergic system functioning. The present study examines age differences in adaptive and coping responses in rats, using both physiological and behavioral indices.

Rationale

Research on the senescent organism has described impairments in both central and peripheral nervous system mechanisms. Behaviorally, these manipulations have been linked to a progressive loss of adaptation (Seyle and Tuchweber, 1976); the range of responses to acute stress is restricted and the exhaustion to chronic stress is facilitated (Frolkis et al., 1972; Dilman et al., 1979; Timiras, 1978). Changes as a function of age have also been documented for basal homeostatic functioning, with reduced catecholamine and opioid levels and compromised functioning of the hypothalamic-pituitary-adrenal axis being reported. As some of these systems are involved in the mediation of the analgesia associated with the administration of morphine or acute exposure to specific environmental stressors in young adult animals (see reviews: Bodnar et al.,1980; Watkins and Mayer, 1982; Terman et al., 1984), it was the aim of the present study to assess whether rats of different ages display differential analgesic and stress related adaptive behaviors. Three analgesic manipulations with different physiological and pharmacological profiles were studied in separate groups of animals: 2DG, CWS and morphine. Dose- response curves for morphine and 2DG, and temperature-response curves for CWS were assessed to determine changes in these analgesic responses as a function of age and of post-injection and post-swim time interval. In addition, a second physiological response for each analgesic manipulation was monitored to determine whether changes in analgesic responses with increase in age are specific to that response, or were part of an overall

developmental change. Therefore, in addition to the analgesia following morphine and CWS, thermoregulatory changes were also assessed, and in addition to the analgesia following 2DG, food intake was monitored.

The previous literature on changes in basal pain thresholds with increase in age has been equivocal, with reports of increases, decreases, and no change. These contradictory findings are partially attributable to a lack of consistency across experiments in the use of pain-inducing stimuli, response measures, subject variables such as weight and gender, as well as to the absence of a clear developmental profile of functional age relationships for these measures. In most cases, restricted "young" vs. "old" comparisons have been used and might have masked age differences. However, by assessing the performance of a number of age groups simultaneously, one can obtain specific information regarding developmental trends and/or changes (Jensen, Messing, Martinez, Vasquez and McGaugh, 1980). Consequently, the present study assessed baseline pain thresholds and analgesic responses across 5 age groups of rats: 4, 9, 14, 19 and 24 months of age. Only females were used so as to reduce variability across conditions; the weight differences with increases in age generally did not exceed 100 grams. In contrast, aging male rats are characterized by greater body weights than cohort matched female rats or younger rats of both sexes; this has been found to contribute to differences in pain responsivity to electrical stimuli, differences in the absorption of drugs, and differences in sensitivity to cold (Pare, 1969; Kato, 1964, Algeri et al., 1982). Furthermore, analgesia was assessed with two pain tests: the tail-flick test,

which measures reactivity to heat (D'Amour and Smith, 1941), and the jump test, which measures reactivity to shock (Evans, 1961). These nociceptive measures act at different levels of the nervous system with tail flick spinally mediated, and jump thresholds mediated supra-spinally.

Although research on the aged animal has revealed changes in endocrine functioning with change in age, e.g., glucose-insulin responsivity , information is lacking regarding the effects of glucoprivation on behavior. Therefore, in the first experiment, we assessed the effects of 2DG on analgesia in five age groups, using a wide range of doses (0, 50, 250, 450 and 650 mg/kg), and several postinjection intervals (30, 60 and 120 minutes). To further elucidate developmental differences in reactivity to stress elicited by glucoprivation, the degree of hyperphagia induced by varying dosages of 2DG was assessed. In the second experiment, the effects of CWS stress was examined, not only in terms of age related alterations in thermal responsivity, but also in terms of analgesia. By using a variety of water temperatures, pain response measures, and post-swim intervals, a developmental profile of adaptive coping responses was obtained. Finally, in the third experiment, to assess age differences in responsivitiy to morphine, analgesia was assessed on 2 pain response measures, across a variety of doses (0, 1.0, 2.5, 5.0, 10.0), and post-injection time intervals (30, 60, 90 and 180 min). The concommitant assessment of core body temperature was aimed at a determination of whether there are age related differences in pain inhibitory systems and/or in general systems that mediate adaptive and coping behavior in rats.

GENERAL METHOD

Subjects

Subjects were 128 female albino Sprague Dawley rats, distributed across five age cohorts: 4 months (n=30), 9 months (n=30), 14 months (n=24), 19 months (n=24), and 24 months (n=20) at the time of testing. Fifty animals were purchased from Sasco Inc.(Omaha, NE), while the remainder (78) were bred and raised in the Queens College vivarium facility. However, each age group had representative animals from both Sasco and the breeding facility, which consisted of a mixture of retired breeders and virgins. All animals were maintained in the same laboratory environment for a minimum of 3 weeks prior to testing. They were housed in pairs in wire mesh cages, maintained on a 12 hour light: 12 hour dark cycle, and allowed ad libitum access to Purina rat chow and water.

Apparatus:

Tail Flick Test: A radiant heat source (IITC Company) was mounted 8 cm dorsal and 4 cm proximal to the tail of a lightly-restrained rat. The switching-on of the radiant heat producing beam started a digital timer. When the animal flicked its tail, a photocell ciruit was activated, stopping the timer which displayed the tail flick latency to the nearest 1/100th of a second.

Jump Test: Electric shocks were delivered to the 16 grids of a 30 cm by 24 cm floor, through a 60 Hz constant shock generator (BRS/LVE) and grid scrambler (Campden Instruments). The footshocks were of a 300 msec duration, and the current intensities were increased until the jump threshold was obtained.

Core Temperature: Rectal temperatures were obtained by inserting a probe of a digital thermometer (Bailey Instruments) which was held in place until a stable reading (0.1°C) was obtained.

Procedure: Pre-Experimental Manipulation

As each animal entered its appropriate age cohort, tail flick latencies and jump thresholds were assessed over a 7 day period until four stable baseline values were obtained. The order of tail flick tests before jump tests was used because this sequence minimizes carryover effects (Kelly, 1982). Each testing session consisted of 3 tail flick latency determinations, made at 15 second intervals. A low intensity beam as established in previous studies (D'Amour and Smith, 1941) was used so as to elicit stable baseline tail flick latencies between 2.00-3.00 seconds. In order to avoid tissue damage, a predetermined criterion cutoff (of radiant heat) of 6.0 seconds was established.

Following the assessment of tail flick latencies, jump thresholds were determined. An ascending method of limits was employed since suprathreshold shock intensities can produce analgesia (Watkins and Mayer, 1982). The jump threshold was defined as the lowest of 2 consecutive intensities that elicited simultaneous withdrawal of both hindpaws from the grids (msec). Each trial began with the animal receiving a 300 msec footshock at a current intensity of 0.10 mA. Subsequent shocks were increased in equal 0.05 mA steps at 10 sec intervals until the jump threshold was determined. After each trial the current intensity was reset to 0.1 mA for the next trial until six threshold determinations were made. The daily jump threshold was calculated as the mean of 6 trials.

Design

Following the attainment of stable tail-flick latencies and jump thresholds, all rats within a given age group were randomly assigned to either the 2DG, CWS or morphine experiments. There were 44 rats in the 2DG experiment, 42 rats in the CWS experiment, and 42 rats in the morphine experiment. Once assigned to a given experiment, each rat was administered either an ascending series of 2DG doses, a descending series of CWS temperatures, or an ascending series of morphine doses. Consequently, for each experiment, a mixed analysis of variance was performed on the data; age was a between-subjects variable, drug dose/swim condition a within subjects variable, and post-injection/swim intervals a within subject variable.

Statistical Analysis

A split plot analysis of variance was performed to assess main independent group effects (control vs. experimental conditions and age group differences), repeated measure effects (across doses and across post-injection/swim intervals) and interactive effects (between age groups and post-injection/swim intervals, between age groups and doses, between post-injection/swim intervals and doses, and between age groups, doses and post-injection/swim intervals). If statistically significant, the Dunnett procedure ($p = .05$) was applied to determine which age groups displayed analgesia, and across which doses and post-injection intervals. In those cases in which there were baseline differences as a function of age, the data were transformed by calculating difference scores to partial out the baseline variance. Analyses of variance were then performed on the transformed data. If statistically significant analgesia and age

group effects were found, correlational statistics (Pearson Product Moment Correlation; $p = .05$) were used to assess the degree of covariation between age and experimental response measures.

Experiment 1: 2DG

Method

2DG Analgesia

2DG analgesia was assessed for 44 rats in the following 5 age groups: 4mo (n=10), 9mo (n=10), 14mo (n=8), 19mo (n=8), and 24 mo (n=8). The rats were administered to an ascending order of the following 5 dosages of 2DG (Sigma Labs): 0, 50, 250, 450 and 650 (2ml normal saline/kg body weight, IP). These doses these have been established in previous studies (Bodnar, Kelly, Brutus, Mansour and Glusman , 1978). Tail flick latencies and jump thresholds were assessed at 30, 60, and 120 min post-injection intervals. A minimum of 4 days elapsed between dosages to eliminate tolerance effects (Spiaggia, Bodnar, Kelly and Glusman,1979).

2DG Hyperphagia

One week after analgesia testing, all animals were administered each of 3 doses of 2DG: 0, 650, 1200 mg/kg (2ml normal saline/kg body weight, IP) in ascending order. The doses used were based upon previous studies (Bodnar, Kelly, Brutus and Glusman, 1978). A minimum of 4 days elapsed between each dosage. Animals were housed individually in wire mesh cages containing preweighed Purina rat chow pellets. The amount of food consumed was determined by subtracting the remaining food from the preweighed value to determine intake to the nearest 0.1g. Spillage was accounted for by recovering any food residue beneath the cage and subtracting this value from the overall food intake.

Results

Tables 1 through 12 show the mean absolute and mean difference scores, the standard error of the means, and the significant main effects and interactions for tail-flick latencies, jump thresholds, and food intake following 2DG administration. Figures 1 through 7 exhibit the effects of 2DG as a function of age at selected post-injection intervals and doses.

2DG Analgesia (Tail-Flick Test): Figure 1 and Tables 1 and 2 summarize the effects of 2DG administration on the tail-flick test performance of the five age groups. Whereas tail flick latencies of all age groups failed to increase significantly following the 50 mg/kg dose of 2DG, there were significant increases in latency following the 250, 450 and 650 mg/kg doses relative to vehicle injection in most age groups. However, the degree and duration of analgesia on the tail-flick test varied across doses and age groups. For example, the 4-month old group showed significant increases in tail-flick latencies 30 and 60 min following the 250 mg/kg dose, and for the entire 120 min interval following the 450 and 650 mg/kg doses. A similar pattern of significant increases in tail flick latencies was observed for the 9-month age group, except for a shorter latency 120 min following the 650 mg/kg dose. Although the 14 month age group showed the same pattern of significant increases in tail flick latencies as the 2 younger age groups, except for a more persistent analgesia at the 250 mg/kg dose, the latency scores did not reflect the progressive increases at higher doses found for

Table 1. Alterations in Tail-Flick Latencies (sec: SEM) Following 2-deoxy-D-glucose (2DG) across age groups.

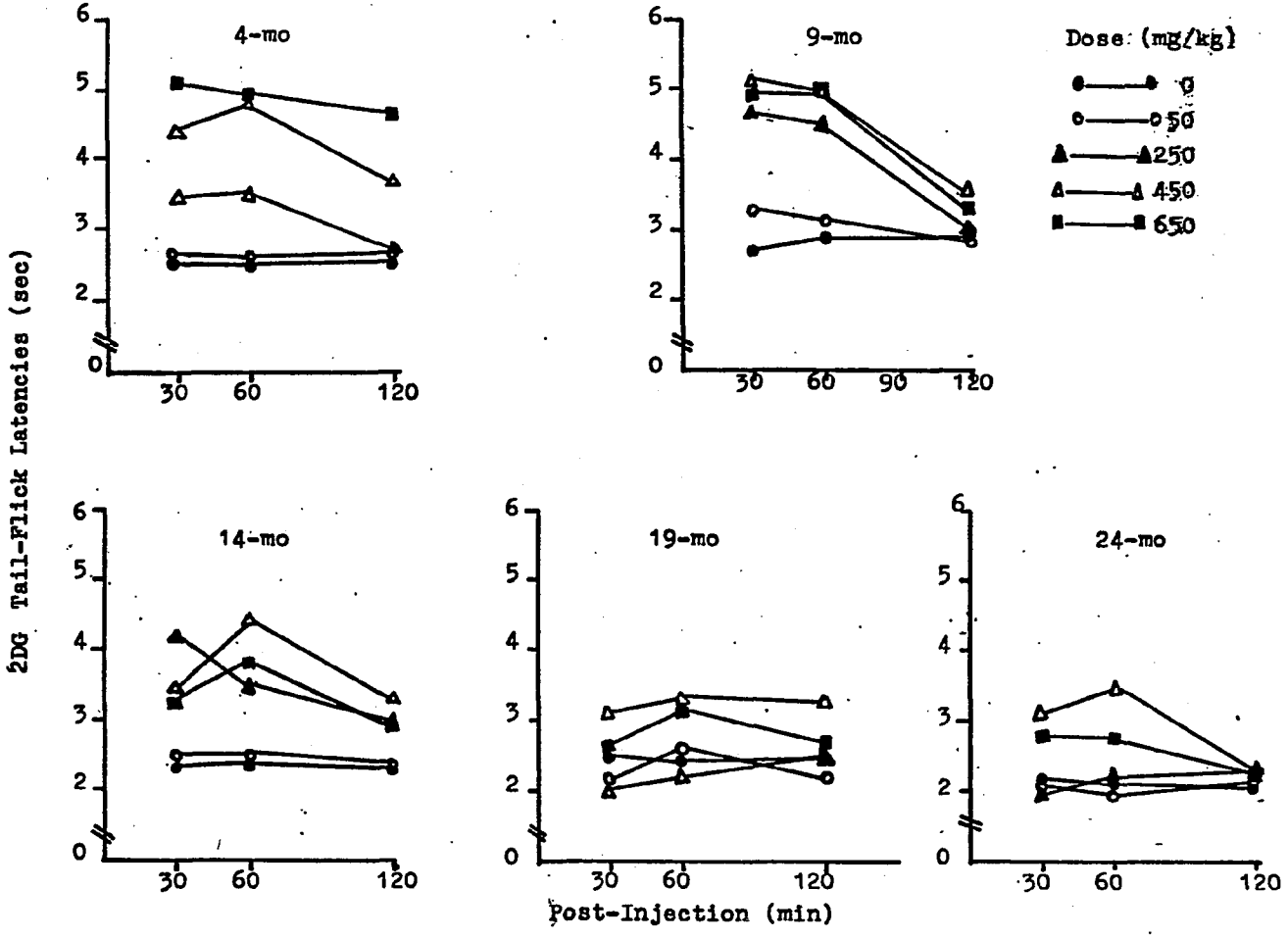
Age Group	2DG Dose (mg/kg)	Post-Injection (min) 30		60		120	
		mean	SEM	mean	SEM	mean	SEM
4-month	0	2.49	(0.08)	2.47	(0.08)	2.57	(0.09)
	50	2.59	(0.18)	2.54	(0.14)	2.64	(0.18)
	250	3.42	(0.32)*	3.48	(0.32)*	2.68	(0.15)
	450	4.41	(0.45)*	4.80	(0.45)*	3.70	(0.36)*
	650	5.14	(0.44)*	4.92	(0.24)*	4.83	(0.35)*
9-month	0	2.75	(0.10)	2.86	(0.12)	2.88	(0.09)
	50	3.28	(0.09)	3.11	(0.15)	2.83	(0.14)
	250	4.63	(0.24)*	4.57	(0.28)*	3.01	(0.10)
	450	5.12	(0.25)*	4.99	(0.28)*	3.60	(0.05)*
	650	4.92	(0.26)*	4.97	(0.19)*	3.37	(0.31)
14-month	0	2.38	(0.12)	2.36	(0.08)	2.25	(0.12)
	50	2.52	(0.18)	2.49	(0.07)	2.34	(0.07)
	250	4.21	(0.43)*	3.48	(0.57)*	3.02	(0.26)*
	450	3.45	(0.32)*	4.42	(0.41)*	3.31	(0.25)*
	650	3.22	(0.45)*	3.78	(0.50)*	2.89	(0.36)*
19-month	0	2.53	(0.08)	2.49	(0.10)	2.42	(0.06)
	50	2.20	(0.08)	2.60	(0.15)	2.20	(0.12)
	250	2.04	(0.12)	2.19	(0.11)	2.51	(0.17)
	450	3.10	(0.35)*	3.29	(0.44)*	3.13	(0.45)*
	650	2.66	(0.50)	3.08	(0.45)*	2.63	(0.14)
24-month	0	2.20	(0.13)	2.11	(0.08)	2.05	(0.13)
	50	2.06	(0.11)	1.92	(0.08)	2.09	(0.09)
	250	2.04	(0.07)	2.21	(0.10)	2.20	(0.09)
	450	3.10	(0.35)*	3.51	(0.08)*	2.35	(0.11)
	650	2.82	(0.25)*	2.76	(0.24)*	2.40	(0.11)

Note: * Significant differences ($p<.01$, Dunnett comparison) from corresponding vehicle value.

TABLE 2: Summary of the Analysis of Variance for Tail Flick Latencies following 2-deoxy-D-glucose (2DG) across Age Groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	189.99	4	47.50	15.49	<.001
Dose (D)	204.10	4	51.02	65.35	<.001
Time (T)	28.84	2	14.42	57.37	<.001
A x D	86.35	16	5.40	6.91	<.001
A x T	15.95	8	1.99	7.93	<.001
D x T	17.30	8	2.16	7.87	<.001
A x D x T	20.79	32	0.65	2.37	<.001

Figure 1. Alterations in tail-flick latencies during the 120 min interval following 2-deoxy-D-glucose (2DG) administration in different age groups.


4-mo
9-mo
14-mo
19-mo
24-mo
2DG Tail-Flick Latencies (sec)
Post-Injection (min)
Dose (mg/kg)
0
50
250
450
650

the younger age groups. Both the 19 and 24-month age groups failed to display significant increases in tail-flick latencies at the 250 mg/kg dose. The 19-month age group did show an increase in tail flick latencies at all post-injection intervals for the 450 mg/kg dose, and for up to 60 min following the 650 mg/kg dose. The 24-month age group showed increased latencies for up to 60 min following both the 450 and 650 mg/kg doses. Thus, regarding the duration of 2DG analgesia, no apparent pattern emerged across age groups, although all age groups showed their maximum analgesic effect for up to 60 min following 2DG administration. However, the data suggest that the degree of analgesia on the tail flick test among age groups does vary as a function of the 2DG dose administered. Whereas the 4-month group displayed a progressive systematic increase in tail flick latencies with increasing 2DG dose, the peak analgesic effect was reached and then maintained at the 450 and 250 mg/kg doses for the 9 and 14-month age groups respectively. In contrast, the two oldest age groups only displayed analgesia at the higher doses.

Since tail flick latencies following the vehicle injection differed significantly among age groups (the 14, 19 and 24-month groups displayed significantly lower tail-flick latencies than the 9-month group), a difference score analysis of variance was performed to ascertain whether the magnitude of 2DG analgesia differed among rats of different ages. Figure 2 and Tables 3 and 4 summarize the differences among age groups relative to the 4-month group for each 2DG dose. Although the 9-month age group only showed lower tail-flick latencies at 60 and 120 min following the 650 mg/kg dose, the 14, 19 and 24-month age groups showed this reduction for the

Table 3. Alterations in tail-flick latency difference scores (sec:SEM) as a function of age relative to the 4 month Age Group following 2-deoxy-D-glucose (2DG).

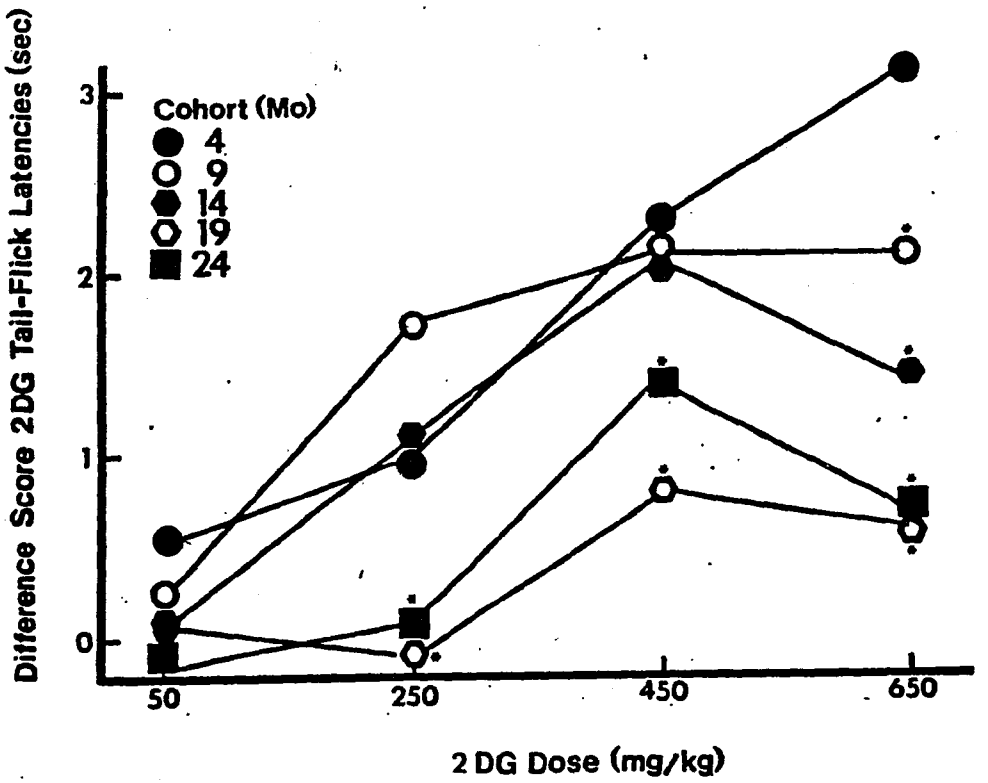
Age Group	2DG Dose (mg/kg)	Post-Injection (min) 30 mean	SEM	60 mean	SEM	120 mean	SEM
4-month	50	.089	(0.14)	.054	(0.17)	.068	(0.16)
	250	.939	(0.30)	1.014	(0.31)	.113	(0.15)
	450	1.882	(0.42)	2.333	(0.38)	1.130	(0.32)
	650	2.655	(0.40)	3.171	(0.23)	2.261	(0.33)
9-month	50	.529	(0.13)	.251	(0.12)	-.065	(0.14)
	250	1.874	(0.24)*	1.711	(0.23)	.129	(0.12)
	450	2.373	(0.26)	2.134	(0.25)	.723	(0.40)
	650	2.179	(0.29)	2.115	(0.17)**	.486	(0.29)**
14-month	50	.140	(0.14)	.134	(0.12)	.091	(0.12)
	250	1.831	(0.38)	1.149	(0.58)	.766	(0.22)
	450	1.070	(0.27)**	2.069	(0.40)	1.057	(0.22)
	650	.834	(0.42)**	1.420	(0.53)**	.641	(0.31)**
19-month	50	-.325	(0.10)	.110	(0.18)	-.216	(0.16)
	250	-.102	(0.34)	-.170	(0.13)**	.094	(0.20)
	450	.567	(0.41)**	.804	(0.46)**	.715	(0.12)
	650	.130	(0.17)**	.577	(0.12)**	.215	(0.18)**
24-month	50	-.140	(0.12)	-.192	(0.12)	.039	(0.15)
	250	-.131	(0.12)**	.101	(0.13)**	.142	(0.12)
	450	1.146	(0.31)**	1.400	(0.20)**	.296	(0.17)**
	650	.627	(0.27)**	.647	(0.28)**	.349	(0.13)**

Note: significant increases (*) or decreases (**) in tail flick latencies are compared with the corresponding 2DG score of the 4 mo age group. ($p<.05$, Dunnett comparison).

TABLE 4: Summary of the Analysis of Variance for Tail Flick Latency Difference Scores following 2DG

Condition	SS	df	MS	F-value	p
Age Group (A)	104.80	4	26.20	8.80	<.001
Dose (D)	136.97	3	45.66	51.66	<.001
Time (T)	33.28	2	16.64	42.19	<.001
A x D	63.54	12	5.29	5.99	<.001
A x T	27.98	8	3.50	8.87	<.001
D x T	11.54	6	1.92	5.40	<.001
A x D x T	13.52	24	0.56	1.58	<.050

Figure 2. Alterations (*- Dunnett comparison) in tail-flick latencies at 60 min following 2DG in different age groups. The difference scores, derived by subtracting the vehicle latencies from the latencies elicited 60 min following each 2DG dose for each group, were calculated because significant differences in vehicle latencies were observed among age groups.


Difference Score 2DG Tail-Flick Latencies (sec)
Cohort (Mo)
4
9
14
19
24
3
2
1
0
50
250
450
650
2 DG Dose (mg/kg)

entire 120 min interval. The 19-month age group also showed significantly lower tail flick latencies 30 and 60 min following the 250 and 450 mg/kg doses, and the 24-month age group displayed lower latency scores for up to 60 min following the 250 mg/kg dose and up to 120 min following the 450 mg/kg dose. This suggests that with increase in age, the degree and duration of tail-flick latencies became progressively lower compared to the 4-month group. Moreover, whereas the 9 and 14-month groups primarily showed this reduction at the higher 2DG doses, the 19 and 24-month ag e groups showed reduced tail flick latencies following all but the lowest dose.

The magnitude and rate of the age-related decline in 2DG latency on the tail-flick test were examined by correlating the age with total latency difference score, which was determined by collapsing difference scores across test times for each animal at each dose. Significant negative correlations between age group and latency scores were found following the 250 ($r=-.458$; $r^2=.210$, $p<.01$), 450 ($r=-.422$; $r^2=.178$, $p<.01$), and 650 mg/kg ($r=-.690$; $r^2=.480$, $p<.01$) doses. Significant variance was accounted for at all of these doses, and was most pronounced at the highest dose. Thus it appears that 2DG analgesia on the tail flick test decreases with increase in age.

2DG Analgesia (Jump Test): Figure 3 and Tables 5 and 6 summarize the alterations in jump thresholds following 2DG administration across age groups. As in the tail-flick test, none of the age groups displayed significant change in jump threshold following the 50 mg/kg dose of 2DG. In contrast, age differences were found for the 250, 450 and 650 mg/kg doses. The 4-month group exhibited significant increases in jump thresholds 30 and 60 min

Table 5: Alterations in jump thresholds (mA: SEM) following 2-deoxy-D-glucose (2DG) across age groups.

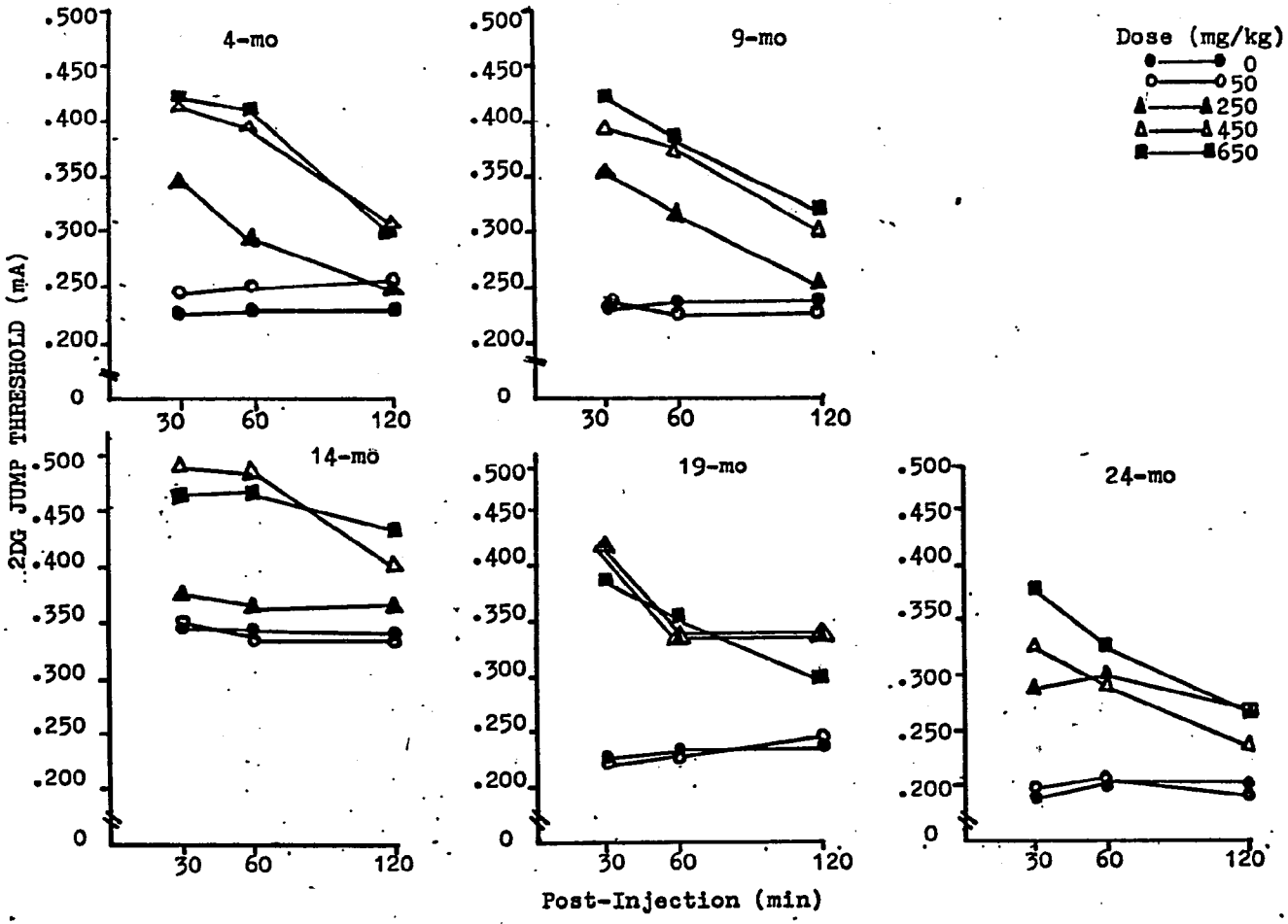
Age Group	2DG Dose (mg/kg)	Post-Injection (min) 30 mean SEM	60 mean SEM	120 mean SEM
	0	.228 (.009)	.231 (.005)	.233 (.008)
4-month	50	.248 (.008)	.252 (.008)	.257 (.006)
	250	.348 (.011)*	.293 (.009)*	.255 (.011)
	450	.413 (.012)*	.392 (.015)*	.308 (.013)*
	650	.418 (.015)*	.410 (.020)*	.303 (.010)*
9-month	0	.233 (.006)	.236 (.007)	.242 (.009)
	50	.233 (.010)	.229 (.009)	.237 (.005)
	250	.353 (.016)*	.316 (.008)*	.253 (.007)
	450	.391 (.009)*	.380 (.016)*	.302 (.011)*
	650	.422 (.016)*	.387 (.014)*	.322 (.013)
14-month	0	.347 (.014)	.344 (.009)	.343 (.010)
	50	.350 (.015)	.334 (.012)	.335 (.012)
	250	.375 (.017)	.363 (.019)	.365 (.018)
	450	.489 (.023)*	.480 (.019)*	.403 (.023)*
	650	.459 (.022)*	.465 (.018)*	.432 (.015)*
19-month	0	.224 (.014)	.232 (.010)	.236 (.012)
	50	.221 (.015)	.230 (.009)	.247 (.018)
	250	.418 (.012)*	.334 (.028)*	.340 (.012)*
	450	.416 (.030)*	.334 (.023)*	.340 (.025)*
	650	.382 (.027)*	.348 (.028)*	.300 (.025)*
24-month	0	.186 (.010)	.199 (.009)	.204 (.010)
	50	.193 (.010)	.204 (.009)	.193 (.006)
	250	.290 (.020)*	.306 (.019)*	.266 (.024)*
	450	.326 (.026)*	.288 (.015)*	.233 (.018)
	650	.370 (.026)*	.324 (.032)*	.264 (.006)*

Note : * Significant difference ($p<.01$, Dunnett comparison) from corresponding vehicle value.

TABLE 6: Summary of the Analysis of Variance for Jump Thresholds Following 2DG Across Age Groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	1.17	4	0.30	27.00	<.001
Dose (D)	1.92	4	0.48	208.79	<.001
Time (T)	0.25	2	0.12	91.83	<.001
A x D	0.14	16	0.08	3.85	<.001
A x T	0.23	8	0.00	2.13	<.050
D x T	0.22	8	0.03	26.03	<.001
A x D x T	0.09	32	0.00	2.69	<.001

Figure 3. Alterations in jump thresholds during the 120 min interval following 2DG administration in different age groups.


4-mo
9-mo
14-mo
19-mo
24-mo
Dose (mg/kg)
0
50
250
450
650
2DG JUMP THRESHOLD (mA)
.500
.450
.400
.350
.300
.250
.200
0
30
60
120
Post-Injection (min)

following the 250 mg/kg dose, and for 120 min following the 450 and 650 mg/kg doses. A similar pattern of increases in jump thresholds were observed for the 9-month age group, except higher jump thresholds were not found at 120 min following the 650 mg/kg dose. In contrast, the 14-month age group failed to show significant increases in analgesia relative to the vehicle at the 250 mg/kg dose, but did show a significant increase in jump thresholds across the time course following the 450 and 650 mg/kg doses. The 19-month age group displayed significant increases in jump thresholds for 120 min following the 250, 450 and 650 mg/kg doses. A similar pattern of significant increases in jump thesholds relative to the vehicle injection was observed for the 24-month age group, although no analgesia was observed 120 min following the 450 mg/kg dose, and jump threshold scores were generally lower at all doses. Thus, regarding the duration of analgesia as measured by performance on the jump test, all age groups show the maximum analgesic effect for up to 60 min following 2DG administration. However, the decline in jump threshold scores between the 60 and 120 min interval was more gradual in the 19-month age group. The degree of analgesia on the jump test among age groups varied as a function of dose as well. Whereas the general trend across all post-injection injection intervals was for the 4, 9 and 14-month age groups to reach peak analgesia at the 450 mg/kg dose and then to maintain it at the 650 mg/kg dose, the 19 and 24- month age groups reached peak analgesia at the 250 mg/kg dose and maintained elevated jump thresholds at the higher doses.

Since jump thresholds following the vehicle injection differed significantly among age groups (with the 14-month and 24-month age

Table 7: Alterations in jump threshold difference scores (mA: SEM) as a function of age relative to the 4 month age group following 2-deoxy-D-glucose

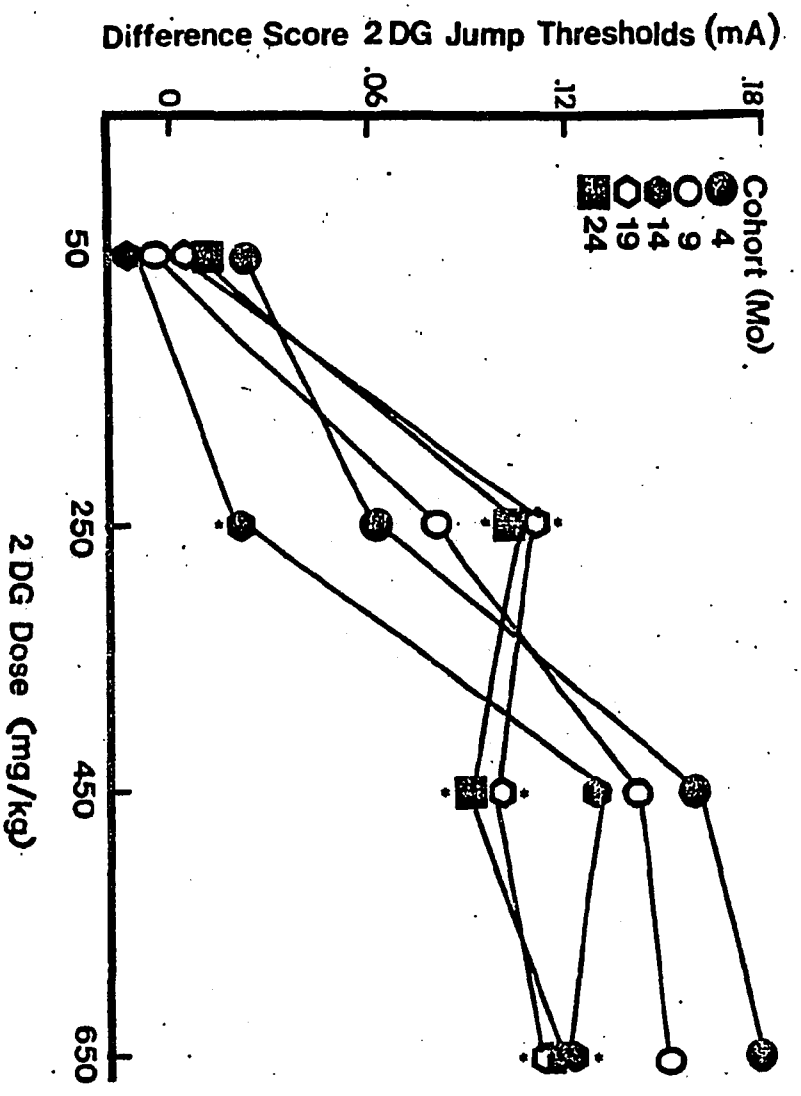
Age Group	2DG Dose (mg/kg)	Post-Injection (min) 30		60		120	
		mean	SEM	mean	SEM	mean	SEM
4-month	50	.020	(.010)	.022	(.007)	.023	(.006)
	250	.120	(.012)	.062	(.012)	.022	(.014)
	450	.186	(.012)	.162	(.015)	.075	(.014)
	650	.193	(.013)	.179	(.019)	.071	(.009)
9-month	50	.000	(.009)	-.007	(.008)	-.005	(.008)
	250	.119	(.017)	.080	(.011)	.011	(.008)
	450	.157	(.009)	.144	(.015)	.060	(.009)
	650	.189	(.016)	.150	(.014)	.080	(.009)
14-month	50	.003	(.017)	-.010	(.017)	-.007	(.018)
	250	.049	(.026)**	.024	(.026)	.010	(.015)
	450	.147	(.022)	.130	(.022)	.052	(.016)
	650	.112	(.028)	.121	(.027)	.090	(.020)
19-month	50	-.003	(.008)	-.002	(.007)	.010	(.010)
	250	.194	(.009)*	.112	(.025)*	.044	(.010)
	450	.192	(.023)	.102	(.017)**	.103	(.017)
	650	.158	(.016)	.116	(.021)**	.063	(.021)
24-month	50	.006	(.012)	.005	(.011)	-.011	(.012)
	250	.103	(.023)	.107	(.022)	.062	(.021)
	450	.141	(.030)**	.089	(.021)**	.029	(.023)
	650	.183	(.027)**	.125	(.030)**	.059	(.011)

Note: Significant increases (*) or decreases (**) in jump thresholds are compared with the corresponding 2DG score of the 4 mo Age Group. ($p<.05$, Dunnett comparison).

TABLE 8: Summary of the Analysis of Variance for Jump Threshold Difference Scores following 2DG

Condition	SS	df	MS	F-value	p
Age Group (A)	0.08	4	0.02	2.49	>.050
Dose (D)	1.24	3	0.41	161.09	<.001
Time (T)	0.45	2	0.23	78.59	<.001
A x D	0.12	12	0.01	3.84	<.001
A x T	0.03	8	0.00	1.42	>.050
D x T	0.14	6	0.02	18.14	<.001
A x D x T	0.08	24	0.00	2.40	<.001

Figure 4. Alterations (*Dunnett comparison) in jump thresholds at 60 min following 2DG in different age groups. A difference score analysis was performed because significant differences in vehicle thresholds were observed among age groups.


Difference Score 2 DG Jump Thresholds (mA)
0
.06
.12
.18
Cohort (Mo)
4
9
14
19
24
2 DG Dose (mg/kg)
50
250
450
650

groups displaying elevated and reduced jump thresholds, respectively), a difference score analysis of variance was performed to ascertain whether the magnitude of 2DG analgesia differed among age groups. Figure 4 and Tables 7 and 8 show the significant differences in 2DG analgesia on the jump test among age groups relative to the 4-month age group. There were no differences in 2DG analgesia between the 4 and 9-month age groups. In contrast, the 14-month age group showed significantly less analgesia 30 min following the 250 mg/kg dose, and the 19 and 24-month groups showed reduced thresholds at the 60 and 30-60 min period following the 450 and 650 mg/kg doses, respectively. In summary, although no clear age-related pattern in 2DG analgesia on the jump test was found at the 250 mg/kg dose, the general trend at the higher doses was for jump thresholds to be reduced in the older age groups relative to the 4-month group.

Correlation coefficients between the animal's age and its total analgesia score for each 2DG dose, were statistically significant following only the 450 mg/kg dose of 2DG ($r=-.304$; $r^2 = .091$, $p<.01$). The correlations for the 250 mg/mg and 650 mg/kg doses approached but did not achieve, statistical significance. Thus, except for the 250 mg/kg 2DG dose, for which small increases in 2DG analgesia were noted in older animals, it appears that 2DG analgesia, as measured by jump threshold, declines with age. However, the effect does not appear to be as robust or as clear-cut as that on the tail-flick test.

2DG Hyperphagia: Figure 5 and Tables 9 and 10 summarize the alterations in food intake following 2DG administration across age groups. The 4 and 9-month age groups significantly increased food

Table 9. Alterations in food intake (g: SEM) following 2-deoxy-D-glucose (2DG) across Age Groups.

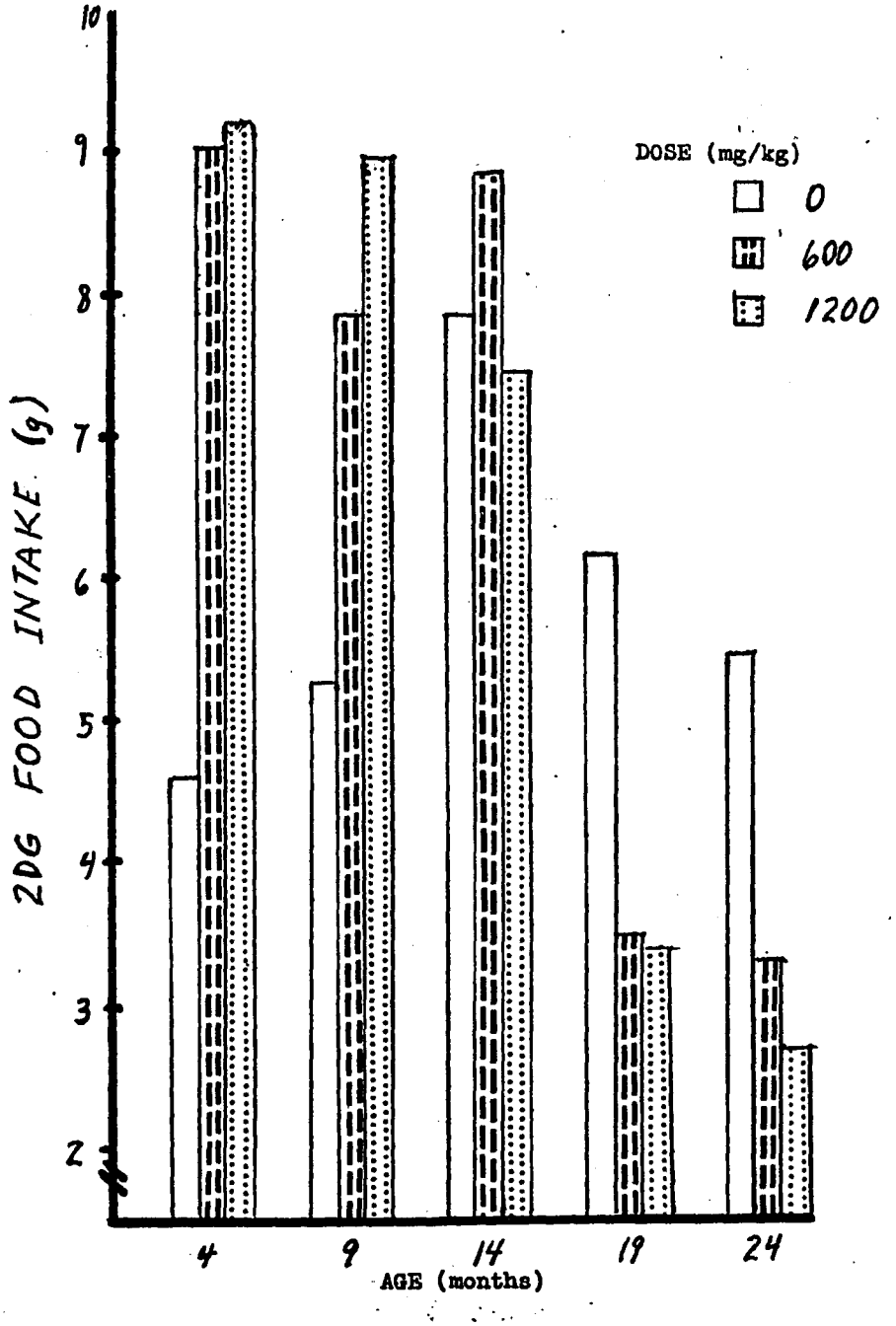
Age Group	2DG Dose (mg/kg)					
	0		650		1200	
	mean	SEM	mean	SEM	mean	SEM
4-month	4.60	(0.29)	9.01	(0.37)*	9.18	(0.42)*
9-month	5.26	(0.39)	7.74	(0.65)*	8.93	(0.59)*
14-month	7.84	(0.80)	8.83	(0.79)	7.43	(0.58)
19-month	6.14	(0.88)	3.50	(0.65)**	3.40	(0.17)**
24-month	5.45	(0.46)	3.31	(0.68)**	2.70	(0.52)**

Note: Significant increases (*) or decreases (**) in food intake are compared with the corresponding vehicle injection ($p<.01$, Dunnett comparison).

Table 10: Summary of the Analysis of Variance for alterations in food intake following 2DG across Age Groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	389.45	4	97.36	22.17	<.001
Dose (D)	9.25	2	4.63	1.99	>.050
A x D	262.94	8	32.87	14.12	<.001

Figure 5. Alterations in food intake at 5 hours following 2DG administration in different age groups.


10
9
8
7
6
5
4
3
2
2DG FOOD INTAKE (g)
DOSE (mg/kg)
0
600
1200
4
9
14
19
24
AGE (months)

intake relative to the vehicle injection following the 650 and 1200 mg/kg doses of 2DG with no differences in the amount of hyperphagia between the two doses. In contrast, there were no changes in food intake following either 2DG dose in the 14-month age group. The 19 and 24-month age groups displayed significant decreases in food intake following the 650 and 1200 mg/kg doses of 2DG as compared to vehicle treatment injection, indicating that with increase in age, 2DG induces hypophagia.

Significant differences were found among age groups in food intake following vehicle treatment with the 14 and 19-month age groups showing greater intake than the 4-month age group. Consequently, a difference score analysis of variance was performed to ascertain whether the magnitude of 2DG hyperphagia or hypophagia differed among age groups. Figure 6 and Tables 11 and 12 show the significant differences in food intake across age groups. Relative to the 4-month age group, the 9-month age group displayed a significant decrease in 2DG hyperphagia which was most pronounced at the 650 mg/kg dose. The 14-month age group also displayed significant decreases in 2DG hyperphagia with maximal differences observed at the 1200 mg/kg dose. The 19 and 24-month age groups were hypophagic relative to all other age groups, an effect that was equivalent for both doses.

To examine the relationship between age and magnitude of decrease in 2DG hyperphagia, age group and 2DG-induced intake difference scores for each animal were correlated. Significant negative correlations between age and food intake were found following the 650 mg/kg ($r=-.77$; $r^2=.59$, $p<.01$) and 1200 mg/kg

Table 11: Alterations in food intake difference scores (g: SEM) following 2-deoxy-D-glucose (2DG) across Age Groups.

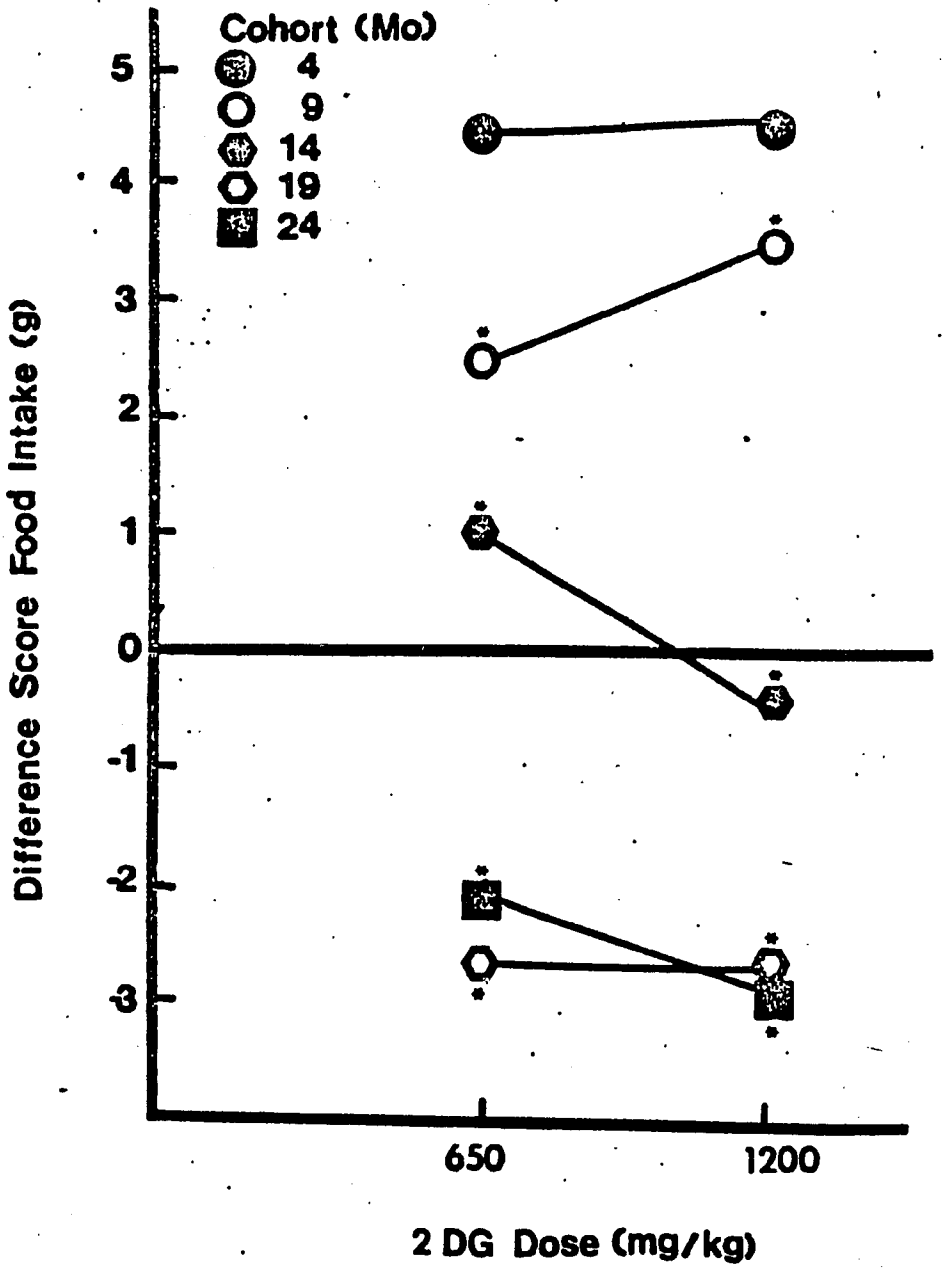
	2DG Dose (mg/kg)			
Age Group	650		1200	
	mean	SEM	mean	SEM
4-month	4.41	(0.45)	4.61	(0.59)
9-month	2.48	(0.79)**	3.67	(0.52)
14-month	0.99	(0.80)**	-0.41	(0.92)**
19-month	-2.64	(1.05)**	-2.66	(0.94)**
24-month	-2.14	(0.74)**	-2.75	(0.73)**

Note : Significant increases (*) or decreases (** in food intake are compared with the corresponding 2DG score of the 4 mo age group ($p<.05$, Dunnett comparison).

Table 12: Summary of the Analysis of Variance for alterations in food intake difference scores following 2DG.

Condition	SS	df	MS	F-value	p.
Age Group (A)	741.57	4	185.39	23.50	<.001
Dose (D)	0.36	1	0.36	0.18	>.050
A x D	16.56	4	4.14	2.03	>.050

Figure 6. Alterations (*Dunnett comparison) in food intake at 5 hours following 2DG in different age groups. A difference score analysis was performed because significant differences in vehicle intake were observed among age groups.


Cohort (Mo)
4
9
14
19
24
5
4
3
2
1
0
-1
-2
-3
Difference Score Food Intake (g)
650
1200
2 DG Dose (mg/kg)

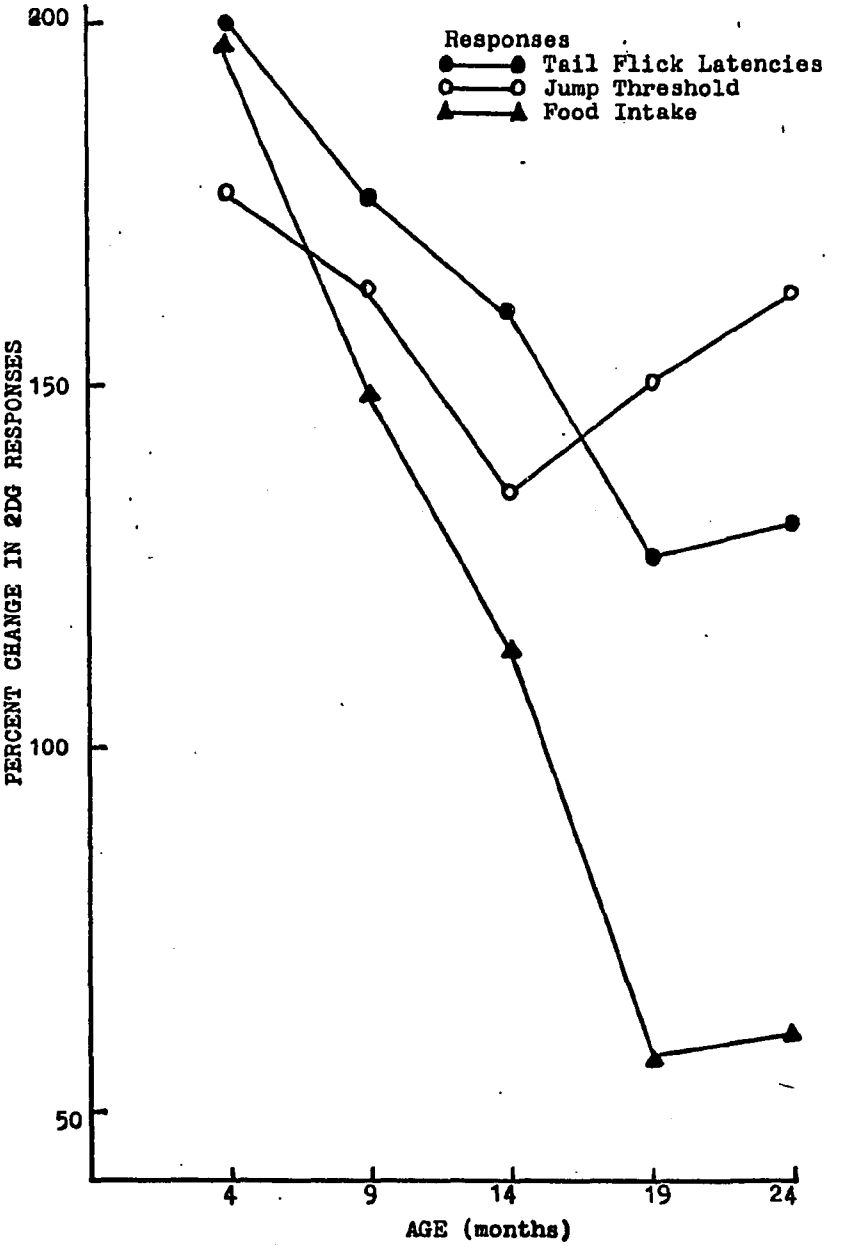
(r=-.801; r^2=.642, $p<.01$) doses of 2DG, indicating that as age increased, the hyperphagic effect of 2DG was initially decreased (9 months), then eliminated (14 months), and then became a hypophagic response (19 and 24 months).

The nature of the relationship between 2DG's effect on food intake and on was examined by correlating the food intake difference score following the 650 mg/kg dose and the score of the same animal on both the tail-flick and jump tests 60 min following the 650 mg/kg dose. The 650 mg/kg dose was selected, as this dose was administered to the same animals in both paradigms. A significant correlation between food intake and analgesia was found only on the tail-flick test (r=.627; r^2=.393, $p<.01$), indicating a consistent pattern of responsivity on tail-flick and food intake measures.

DISCUSSION

An analysis of the results of the 2DG experiment indicates that significant, systematic decreases in the analgesic and hyperphagic responses to 2DG occur as a function of age. Figure 7 displays the percentage change in 2DG responses in regard to tail flick latencies, jump thresholds and food intake following the 650 dose across age groups. For instance, on the tail flick test, 2DG analgesia 60 min following the 650 mg/kg dose was 199% for the 4-month group, 174% for the 9-month group, 160% for the 14-month group, 124% for the 19-month group, and 130% for the 24-month group. This decrease in latency with increase in age accounted for 48% of the variance. Whereas significant decreases in 2DG analgesia occured in older animals on the jump test as well, the age-related decline was not as robust as

Figure 7. Percent change in tail-flick latency, jump threshold and food intake responses 60 min following a 650 mg/kg 2DG dose in different age groups.


Responses
Tail Flick Latencies
Jump Threshold
Food Intake
200
150
100
50
PERCENT CHANGE IN 2DG RESPONSES
4
9
14
19
24
AGE (months)

that found on the tail flick test. However, 2DG hyperphagia showed the same age-related systematic reduction as that observed on the tail flick test. Significant percentage change in food intake observed in the 4 (196%) and 9-month (147)% groups disppeared in the 14-month (113%) group, and was replaced by hypophagia in the 19-month (57%) and 24-month (61%) groups; These effects accounted for 77% - 80% of the variance for the two doses of 2DG employed. Moreover, there was a strong correlation between changes in 2DG analgesia on the tail flick test and changes in 2DG hyperphagia, suggesting that with increases in age, there is a progressive reduction in adaptive responsivity to the physiological stress responses induced by 2DG.

The decrease in analgesia on both tests across age groups occurred independently of age-related changes in baseline pain thresholds, since baseline thresholds were factored out in the difference score analysis. Baseline tail- flick latencies were significantly lower in the 24-month old group relative to the 9-month-old group, significantly higher in the 14- month-old group and significantly lower in the 24-month-old group relative to the 4-month-old group. The hyperresponsivity of the oldest group observed on both nociceptive tests is in accord with some, but not all, previous findings of changes in baseline pain threholds with increasing age (Gordon et al., 1978; Kavaliers et al., 1983; Chan and Lai, 1982; Pare, 1965; Nicak, 1971; Hess et al., 1980). Some of the conflicting findings might be related to the use of different pain-inducing stimuli, different response measures, and/or to a lack of consistency in the species, gender, and ages of the animals used across studies. For example, Spratto and Dorio (1982) reported that

older and younger rats display similar tail flick latencies to a heat stimulus. While this would appear to disagree with the results of the present study, Spratto and Dorio's (1982) young group was 1.5 mo of age, and their old groups 6 and 10 months of age. As the present study found no differences between the 4 and 9 month groups in baseline tail flick latencies, the seemingly discrepant results are concordant. Other researchers, using a wider range of ages, report that both rats and mice display decreased latencies on the hot plate test with increase in age (Chan and Lai, 1982; Kavaliers et al., 1976). Although these findings agree with those of the present study, direct comparisons are difficult because the mechanisms underlying pain sensitivity on the hot plate test differ from those mediating the tail-flick test (Kuraishy, et al., 1983). Similar controversy exists regarding changes in baseline reactivity to an electrical stimulus. For example, Pare (1969) reported that older male rats (14 months) display higher aversive threhsolds for grid shock on a spatial preference task than either age group-matched females or younger rats of both sexes (1 mo, 3 mo, 6 mo). In contrast, reductions in shock thresholds were found as a function of age (3 mo, 12 mo, 18 mo) for both sexes when tail-flick or orienting responses were the response measures (Gordon et al.,1978). The results of the present study are difficult to compare to these findings because jump, flinch and orienting responses are mediated by different mechanisms (Bonnet and Peterson, 1975). However, in accord with Pare's (1969) observations, the 14-month age group did show elevated jump thresholds relative to the 9 month age group, but this tendency for a reduction in pain sensitivity was not manifested in

the two older groups. Instead, the 24-month animals showed a reduction in pain threshold. This is in accord with the results of Gordon and colleagues (1978) who found that older rats (18 mo) had lower flinch and orienting thresholds. Thus, if age changes in pain sensitivity are to be understood, there is a need for assessments of age groups at several ages of the full life span; merely comparing "young" to "old" groups is inadequate.

Changes in pain sensitivity with increases in age are also species dependent. In regard to pain sensitivity levels in elderly humans, both equivalent (Hardy, Wolff, and Goodell, 1943) and higher pain thresholds (Schluderman and Zubek, 1962; Sherman and Robillard, 1960) have been reported compared to younger cohorts. However, other studies suggest that the less efficient detection of both radiant heat and electrical stimuli are limited to suprathreshold stimuli, and differences with increase in age may primarily be attributed to response criterion factors (Clark and Mehl, 1971; Harkins and Chapman, 1976).

The mediation of 2DG analgesia involves both opioid and nonopioid systems. Evidence for opioid system involvement has been derived from the observations that 2DG analgesia displays tolerance, partial cross-tolerance and synergy with morphine (Spiaggia et al, 1979; Bodnar et al., 1983; Bodnar et al., 1978). Given opioid involvement, the present results might be explained in terms of diminished opiate system responsivity, as evidenced by reductions in the level and receptor binding of beta-endorphin, met-enkephalin and leu- enkephalin with increase in age (Jensen et al., 1980; Dupont et al., 1981; Barden et al., 1981; Gambert et al., 1980). However, 2DG

analgesia is not exclusively modulated by an opioid system, since nalaxone fails to affect 2DG analgesia (Bodnar et al., 1979). A variety of other essentially nonopioid systems have been shown to modulate 2DG analgesia as well. A role for the hypothalamo-hypophyseal-adrenal axis has been proposed since 2DG analgesia is potentiated by either hypophysectomy or destruction of the medial-basal hypothalamus in young adult rodents (Badillo- Martinez et al., 1984; Bodnar et al., 1979). Therefore, our findings of a reduction in 2DG analgesia with increase in age may also be related to increased thresholds of the hypothalamus to feedback regulation in its interactions with the pituitary and adrenal cortex (Dilman et al, 1979). For example, older rodents show an impaired ability to increase ACTH levels when exposed to stress (Tang and Phillips, 1978; Britton et al., 1975). A role for catecholamines in mediating 2DG analgesia is also apparent since 2DG analgesia is decreased following pretreatment with the dopamine receptor stimulant apomorphine, and increased following pretreatment with dopamine receptor antagonists (Bodnar et al., 1980; Bodnar and Nicotera, 1982). The aging rodent appears to display inadequate functional responses of catecholamine systems. Reductions in both the levels and turnover rates, as well as in the receptor binding of dopamine and norepinephrine, and in their synthesizing enzymes have been reported for the whole brain, median eminence, pituitary, hypothalamus, striatum and cortex (Finch, 1976; Jonec and Finch, 1975; Timiras et al, 1980; Miller, 1976; Sun, 1976; Ponzio, 1978; Estes and Simpkins, 1980; Govoni et al, 1977, 1980). These effects are accentuated in response to stress. For example, the exposure of older rodents to immobilization stress results in a delay

in the release of norepinephrine, a reduction in beta-noradrenergic responses and prolonged activation of noradrenergic neurons in the hypothalamus, amygdala and pons-medulla (Ida et al., 1982; 1984; Chiuen et al., 1980). Consequently, inadequate functioning of and modulation by catecholamines may explain age changes in analgesic responsiveness.

The systematic, progressive decreases in 2DG hyperphagia as a function of age occurred independently of any significant differences in baseline food intake. The age-related decline in 2DG hyperphagia appears to parallel the progressive glucose intolerance observed with increasing age which has been attributed to impaired glucose metabolism (Reaven et al., 1979) and reduced insulin responsivity by pancreatic islets (Kitahara, et al., 1979). However, 2DG hyperphagia in the young adult rodent appears to be relatively independent of cellular glucoprivation induced by 2DG, as glucoprivically initiated feeding persists in rodents infused with glucose or deprived of food (Ritter et al., 1978; 1981). Rather, a central mechanism of action may mediate 2DG hyperphagia. Again, the age-related reductions in 2DG hyperphagia found in the present study may be explained by changes in opioid or catecholamine systems. Opioid modulation in 2DG hyperphagia is suggested by the reduction in this response following nalaxone pretreatment (Lowy, Maickel and Yim, 1980). Thus, reduced opiate receptor binding and levels of beta-endorphin, met-enkephalin and leu-enkephalin in the hyothalamus, striatum, thalamus, and cortex of aging rodents (Barden et al, 1981; Gambert et al., 1980, Messing et al., 1980) may contribute to the progressive reduction in 2DG hyperphagia. In this regard, older (22 and 28 month) rats are

significantly less sensitive (10-100x) than younger rats (2 and 12 month) to the hypophagic properties of nalaxone and the hyperphagic properties of the kappa-sigma opiate receptor agonist, butorphanol (Gosnell et al, 1983).

Catecholamines are another modulator of 2DG hyperphagia: whereas inteference with dopamine availability reduces 2DG hyperphagia (Stricker and Zigmond, 1974; Berthoud and Mogenson, 1977), 2DG administration blocks norepinephrine depletions and reduces hyperphagic responses following acute foot shock (Ritter et al, 1978; Ritter and Ritter, 1977). Again, the reports of age-related reductions in catecholamine availability and responsivity, particularly in the hypothalamus (Miller, 1976; Jonec and Finch, 1975), may contribute to the reduction in feeding behavior in older animals. Finally, whereas 2DG analgesia and 2DG hyperphagia mechanisms have typically been found to be dissociated, the present results, by providing the first instance in which both effects were reduced similarly may indicate a seperate, independent reduction in the functioning of disparate systems with increase in age.

Experiment 2: CWS

Method

CWS analgesia and hypothermia were assessed for 42 rats in the following five age groups: 4mo (n=10), 9mo (n=10), 14mo (n=8), 19mo (n=8), 24mo (n=6). Following the determination of baseline tail flick latencies and jump thresholds, all animals were exposed to a no swim condition followed by a descending range of water temperatures over a period of 4 weeks, so as to avoid adaptation effects. The water temperatures used - 21°C, 15°C, 8°C, and 2°C - were based upon previous studies (Bodnar, Kelly and Glusman, 1978). Tail-flick latencies and jump thresholds were assessed 30, 60 and 90 minutes following each of the swim conditions (water temperatures). Core body temperatures were measured immediately before and after swims and at 30, 60, and 90 minute post-swim intervals.

Results

Tables 13 through 20 show the mean absolute and mean difference scores, standard error of the means, and significant main effects and interactions for tail-flick latencies, jump thresholds, and core body temperatures following CWS exposure. Figures 8 through 14 show the effects of CWS as a function of age, at selected post-swim intervals and swim conditions.

CWS Analgesia (Tail-Flick Test): Figure 8 and Tables 13 and 14 summarize the alterations in CWS analgesia as measured by

Table 13. Alterations in tail-flick latency scores (sec:SEM) following cold water swim (CWS) across age groups.

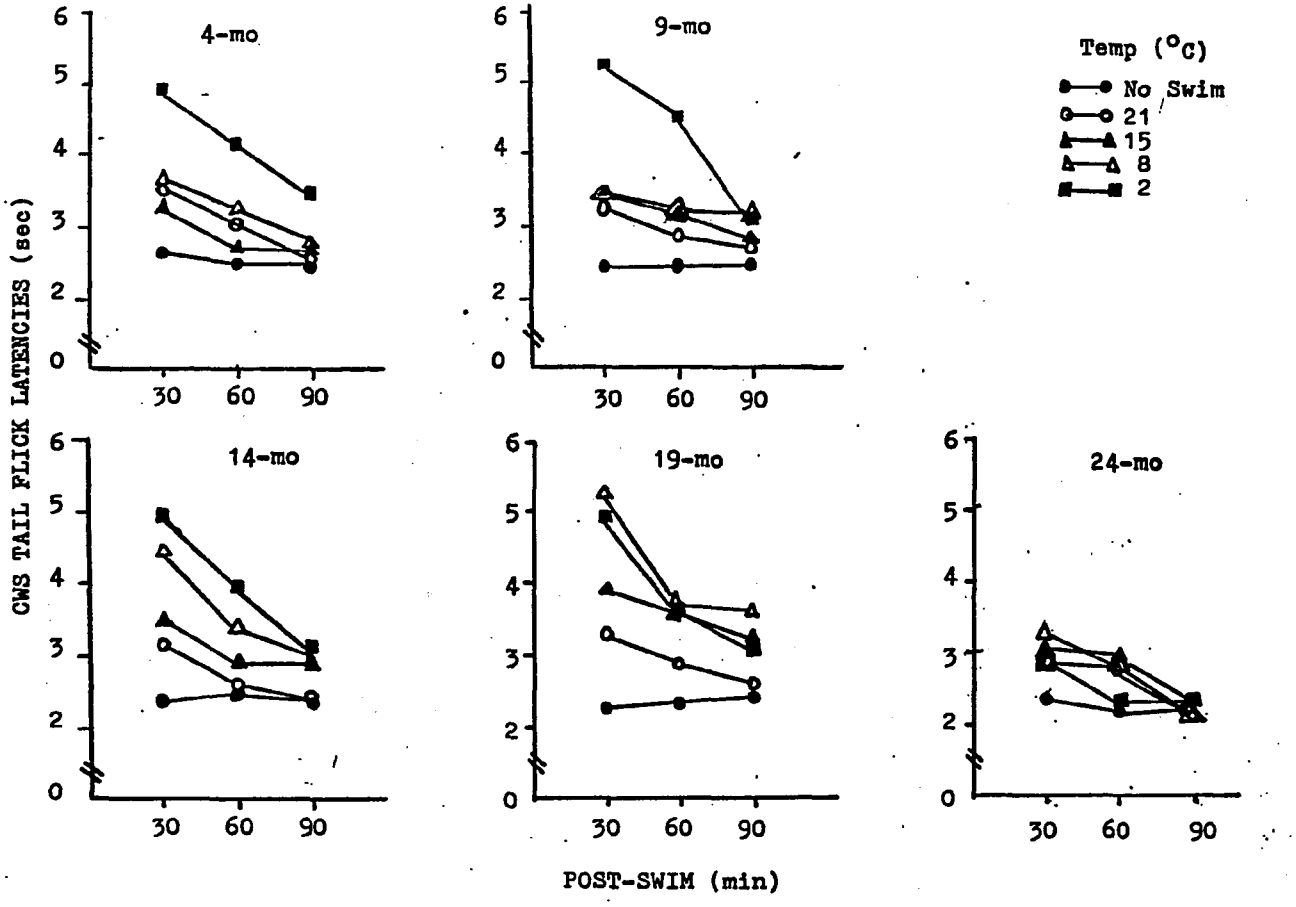
Age Group	Swim Condition (°C)	Post-Injection (min) 30 mean	SEM	60 mean	SEM	90 mean	SEM
4-month	No Swim	2.62	(0.09)	2.48	(0.08)	2.48	(0.09)
	21	3.49	(0.14)*	3.05	(0.11)	2.54	(0.07)
	15	3.33	(0.19)*	2.78	(0.11)	2.65	(0.07)
	8	3.72	(0.20)*	3.32	(0.26)*	2.75	(0.15)
	2	4.88	(0.35)*	4.04	(0.39)*	3.45	(0.18)*
9-month	No Swim	2.42	(0.08)	2.49	(0.08)	2.42	(0.08)
	21	3.28	(0.20)*	2.87	(0.10)	2.74	(0.12)
	15	3.49	(0.15)*	3.27	(0.11)*	2.82	(0.12)
	8	3.42	(0.24)*	3.35	(0.14)*	3.16	(0.16)*
	2	5.22	(0.38)*	4.52	(0.30)*	3.10	(0.18)*
14-month	No Swim	2.38	(0.12)	2.49	(0.09)	2.37	(0.06)
	21	3.15	(0.33)*	2.58	(0.20)	2.38	(0.13)
	15	3.50	(0.21)*	2.91	(0.01)	2.82	(0.22)
	8	4.54	(0.08)*	3.40	(0.05)*	2.88	(0.14)
	2	4.94	(0.11)*	3.91	(0.32)*	3.07	(0.30)*
19-month	No Swim	2.31	(0.07)	2.37	(0.10)	2.39	(0.12)
	21	3.32	(0.36)*	2.93	(0.22)	2.61	(0.11)
	15	3.91	(0.14)*	3.61	(0.32)*	3.25	(0.29)*
	8	5.29	(0.33)*	3.63	(0.35)*	3.59	(0.45)*
	2	4.89	(0.32)*	3.64	(0.22)*	3.02	(0.12)
24-month	No Swim	2.41	(0.10)	2.19	(0.06)	2.23	(0.06)
	21	2.93	(0.26)	2.76	(0.26)	2.14	(0.11)
	15	3.02	(0.08)*	2.99	(0.05)*	2.26	(0.11)
	8	3.25	(0.21)*	2.95	(0.11)*	2.17	(0.06)
	2	2.79	(0.19)	2.34	(0.05)	2.27	(0.05)

Note: * significant differences ($p<.05$, Dunnett comparison) from corresponding vehicle value.

Table 14: Summary of the Analysis of Variance for Tail-Flick Latencies Following CWS Across Age Groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	37.00	4	9.25	8.60	<.001
Swim Cond. (D)	129.22	4	32.30	61.13	<.001
Time (T)	71.47	2	35.74	136.07	<.001
A x D	41.00	16	2.56	4.85	<.001
A x T	3.85	8	0.48	1.83	>.050
D x T	25.94	8	3.24	10.51	<.001
A x D x T	15.90	32	0.50	1.61	<.050

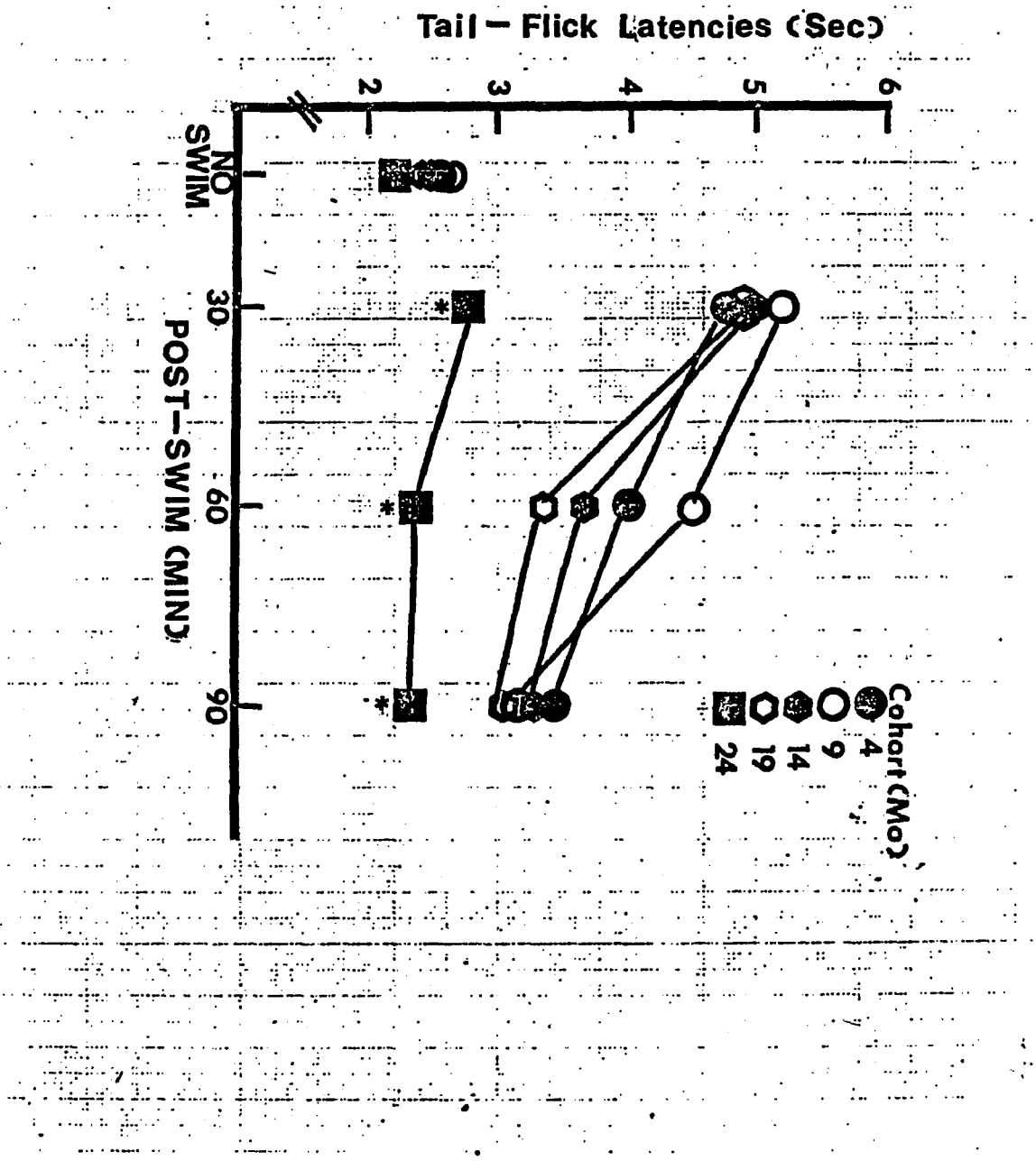
Figure 8. Alterations in tail-flick latencies during the 90 min interval following cold water swims (CWS) in different age groups.


4-mo
9-mo
14-mo
19-mo
24-mo
Temp (°C)
No Swim
21
15
8
2
CWS TAIL FLICK LATENCIES (sec)
POST-SWIM (min)
0
2
3
4
5
6
30
60
90

performance on the tail flick test. Relative to the no-swim condition, CWS induced analgesia in all age groups on the tail-flick test, but the pattern of increase in latencies differed as a function of water temperature, test time and age. All but the 24 mo age group showed significant increases in latency 30 min after all swim conditions. Similarly, at the later post-swim intervals, both the 4 and 14 month groups showed significant increases in tail-flick latencies 60 min following the 8 and 2°C swim conditions and 90 min following the 2°C swim. A similar pattern of increased tail flick latencies were observed for the 9 and 19 month age groups, though increases were also observed 60 min following the 15°C swim and 90 min following the 8 and 15°C swim conditions, respectively. However, although the duration of analgesia appears to be similar in the 4 younger age groups, with tail flick latencies showing a progressive decline from 30 to 90 min, the degree of analgesia on the tail flick test across age groups varied as a function of swim condition. The two younger age groups showed the greatest tail flick latencies at the lowest swim temperature, but the maximum degree of analgesia was reached by 8°C and then maintained by 2°C for the 14 and 19-month age groups. In contrast, CWS does not induce analgesia on the tail-flick test at the warmest and coldest water temperatures in the 24-month age group; significant increases in tail flick latencies occurred only up to 60 min following the 15 and 8°C swim conditions.

Since no age differences in tail flick latencies were found in the no-swim condition, difference scores were not computed. Equivalent analgesic responsivity was observed across all age

Figure 9. Alterations (*Dunnett comparison) in tail-flick latencies during the 90 min interval following a 2oC CWS in different age groups.


Tail – Flick Latencies (Sec)
2
3
4
5
6
NO SWIM
30
60
90
POST–SWIM (MIN)
Cohort (Mo)
4
9
14
19
24

groups but the oldest, except for an increase in tail flick latencies in the 14 and 19 month age groups 30 min following the 8°C swim condition. The most consistent age-related pattern emerged at the 2°C swim, Figure 9 shows the age changes in the magnitude of CWS analgesia on the tail flick test across the time course for the 2°C swim. Whereas the 4, 9, 14, and 19-month age groups demonstrated significant analgesia across all post-injection intervals, which was maximal at the 30 min interval and thereafter showed a progressive time related decrease, the 24 month age group showed significantly reduced analgesia at all post-injection intervals.

To examine the relationship between age and magnitude of CWS analgesia, correlations between age and latency score 30 and 60 min following the swim were computed. Significant correlations between age and latency scores were found only following the 2°C swim ($r=-.533$; $r^2=.284$, $p<.01$). Thus, it appears that there is an age-dependent analgesic effect of CWS on the tail flick test that is evidenced only for the most severe - lowest water temperature used.

CWS Analgesia (Jump Test): Figure 10 and Tables 15 and 16 summarize the alterations in jump thresholds following CWS across cohorts. All age groups showed a gradual increase in jump thresholds 30 min following the swim conditions, but different pattens emerged as a function of age and swim condition. The 4 and 9-month age groups showed identical patterns: although no analgesia was found following the 21°C swim, significant increases in jump thresholds were observed across the time course following the 15°C

Table 15: Alterations in jump thresholds (mA: SEM) following cold water swim (CWS) across Age Groups.

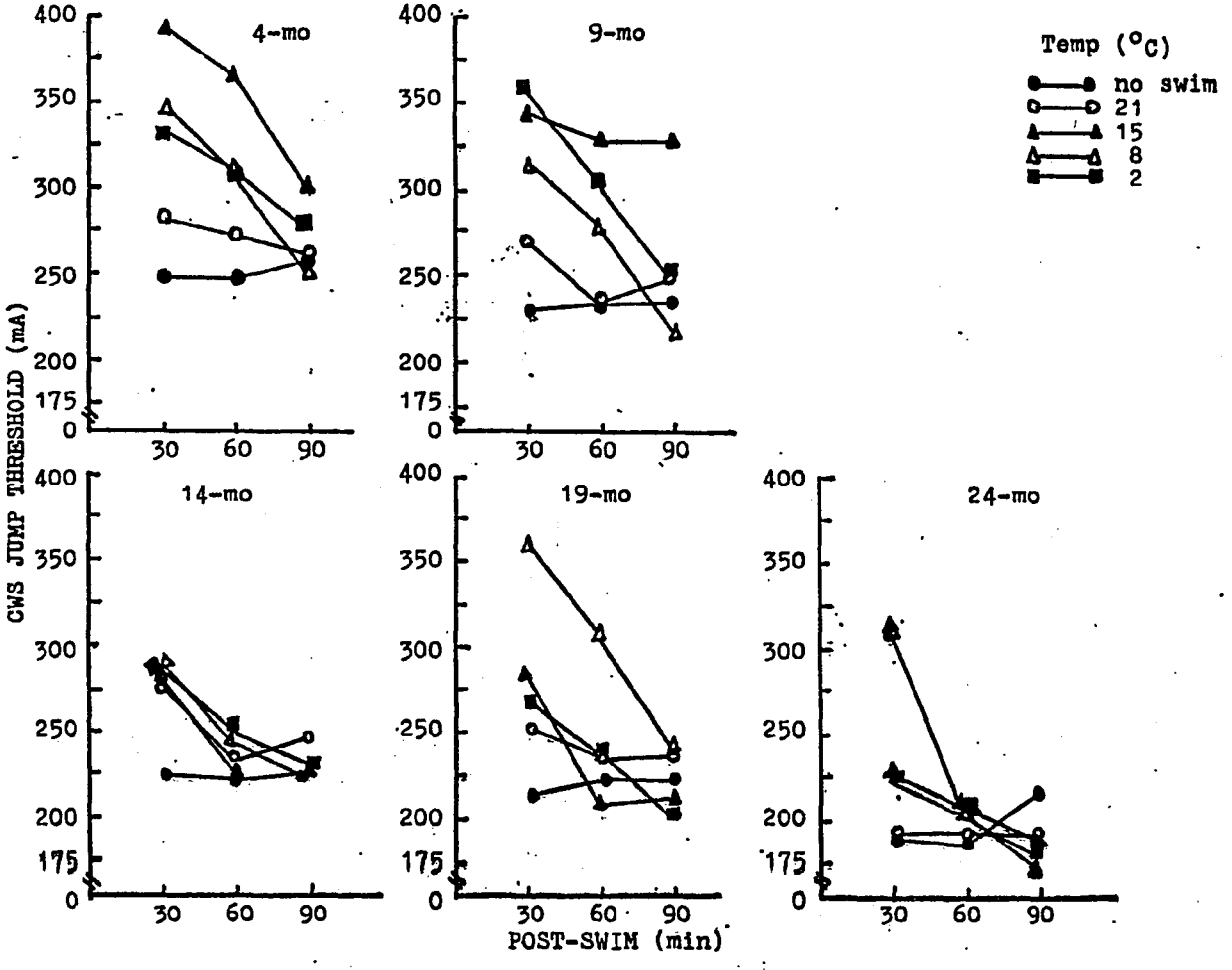
Age Group	Swim Condition (°C)	Post-Injection (min) 30 mean	SEM	60 mean	SEM	90 mean	SEM
4-month	No Swim	.247	(.013)	.248	(.009)	.258	(.014)
	21	.283	(.024)	.273	(.011)	.263	(.009)
	15	.397	(.034)*	.367	(.029)*	.304	(.021)*
	8	.346	(.022)*	.296	(.020)*	.247	(.013)
	2	.333	(.015)*	.307	(.019)	.277	(.014)
9-month	No Swim	.230	(.007)	.233	(.006)	.237	(.008)
	21	.264	(.015)	.234	(.011)	.246	(.010)
	15	.345	(.017)*	.330	(.029)*	.328	(.029)*
	8	.319	(.010)*	.277	(.010)*	.216	(.014)
	2	.361	(.016)*	.301	(.010)*	.245	(.013)
14-month	No Swim	.226	(.012)	.220	(.012)	.228	(.010)
	21	.270	(.013)*	.237	(.009)	.246	(.012)
	15	.286	(.010)*	.228	(.011)	.224	(.012)
	8	.294	(.011)*	.245	(.011)	.225	(.011)
	2	.289	(.016)*	.252	(.011)	.231	(.012)
19-month	No Swim	.215	(.015)	.224	(.016)	.220	(.013)
	21	.254	(.019)*	.237	(.018)	.239	(.014)
	15	.285	(.028)*	.212	(.013)	.210	(.014)
	8	.361	(.046)*	.307	(.035)*	.242	(.020)
	2	.270	(.012)*	.240	(.009)	.199	(.008)
24-month	No Swim	.188	(.011)	.186	(.013)	.208	(.016)
	21	.193	(.011)	.193	(.015)	.192	(.011)
	15	.226	(.008)*	.205	(.012)	.188	(.010)
	8	.312	(.019)*	.204	(.014)	.174	(.007)
	2	.221	(.012)	.208	(.014)	.181	(.007)

Note: * significant differences ($p<.05$, Dunnett comparison) from corresponding vehicle value.

Table 16: Summary of the Analysis of variance for Jump Thresholds Following CWS Across Age Groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	0.56	4	0.14	9.02	<.001
Swim Cond.(D)	0.22	4	0.06	18.00	<.001
Time (T)	0.23	2	0.12	109.92	<.001
A x D	0.25	16	0.01	4.99	<.001
A x T	0.01	8	0.00	0.96	>.050
D x T	0.17	8	0.03	21.07	<.001
A x D x T	0.06	32	0.00	1.78	<.010

Figure 10. Alterations in jump thresholds during the 90 min interval following CWS in different age groups.


4-mo
9-mo
14-mo
19-mo
24-mo
Temp (°C)
no swim
21
15
8
2
CWS JUMP THRESHOLD (mA)
POST-SWIM (min)
400
350
300
250
200
175
0
30
60
90

swim, and 30 and 60 min following the 8 and 2°C swim conditions. As on the tail-flick test, the 14 and 19 month-age groups displayed analgesia at all water temperatures with similar increases noted for both groups 30 min following all swim conditins, although higher jump thresholds were also seen at the 60 min interval following the 15 and 8°C swim conditions, respectively. The 24-month cohort showed a pattern of restricted analgesic responses similar to that observed on the tail-flick test. In contrast to the younger groups (4,9, 14, and 19 mo old), the oldest group did not exhibit CWS analgesia except under 8 and 15 °C at the 30 min post-swim interval.

Since jump thresholds following the no swim condition differed significantly among cohorts, with the 24 month cohort displaying hyperresponsiveness, a difference score analysis of variance was performed to ascertain whether the magnitude of CWS analgesia differed among age groups. Figure 11 and Tables 17 and 18 show the differences in magnitude of CWS analgesia on the jump test among cohorts relative to the 4-month age group. No differences in analgesic responsivity was observed between the 4 and 9-month age groups across swim conditions, except for a more persistent analgesia noted in the 9-month age group after the 15° swim. In contrast, the 14, 19 and 24-month age groups displayed less analgesia across the time course following the 15°C swim relative to the 4 month age group. However, while the 19-month age group displayed greater CWS analgesia 30 min following the 8°C swim condition, the 24-month age group displayed less analgesia 30 min following the 2°C swim. Thus it appears that CWS analgesia on the

Table 17: Alterations in jump threshold difference scores (mA: SEM) as a function of age relative to the 4 month age group following cold water swim (CWS).

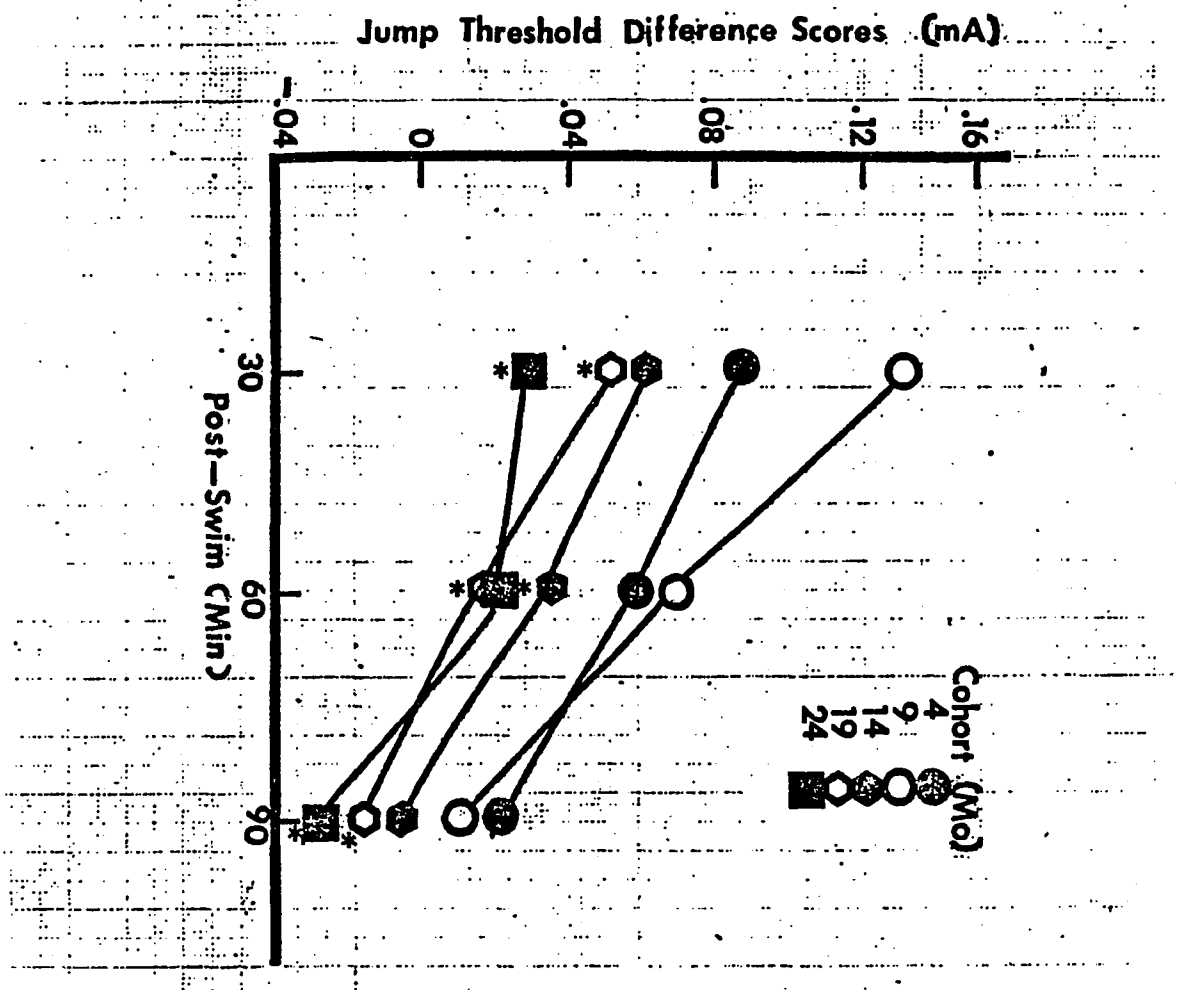
Age Group	Swim Condition (°C)	Post-Injection (min) 30 mean	SEM	60 mean	SEM	90 mean	SEM
4-month	21	.036	(.023)	.040	(.019)	.004	(.016)
	15	.151	(.029)	.119	(.028)	.046	(.022)
	8	.099	(.015)	.048	(.017)	-.011	(.008)
	2	.087	(.013)	.058	(.015)	.020	(.014)
9-month	21	.034	(.011)	.002	(.013)	.009	(.007)
	15	.115	(.016)	.097	(.026)	.092	(.022)*
	8	.089	(.012)	.044	(.010)	-.021	(.011)
	2	.130	(.020)	.068	(.007)	.008	(.012)
14-month	21	.044	(.022)	.017	(.014)	.019	(.022)
	15	.060	(.009)**	.008	(.008)**	-.004	(.011)**
	8	.068	(.015)	.025	(.009)	.011	(.012)
	2	.063	(.021)	.032	(.010)	-.004	(.010)
19-month	21	.038	(.012)	.013	(.008)	.026	(.009)
	15	.070	(.022)**	-.012	(.015)**	-.009	(.016)**
	8	.146	(.037)*	.078	(.029)	.029	(.017)
	2	.054	(.015)	.015	(.010)	-.021	(.011)
24-month	21	.005	(.013)	.004	(.005)	-.017	(.007)
	15	.039	(.015)**	.019	(.007)**	-.021	(.021)**
	8	.124	(.023)	.018	(.012)	-.035	(.016)
	2	.027	(.028)**	.020	(.018)	-.028	(.016)

Note: Significant increases (*) or decreases (**) in tail flick latencies are Compared with the corresponding score of the 4 mo age group. ($p<.05$, Dunnett comparison).

TABLE 18: Summary of the Analysis of Variance for Jump Threshold Difference Scores Following CWS Across Age Groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	0.13	4	0.03	5.32	<.010
Swim Cond.(D)	0.08	3	0.03	7.07	<.001
Time (T)	0.39	2	0.19	77.13	<.001
A x D	0.22	12	0.02	4.87	<.001
A x T	0.02	8	0.00	0.95	>.050
D x T	0.08	6	0.01	10.57	<.001
A x D x T	0.06	24	0.00	1.89	<.010

Figure 11. Alterations (*Dunnet comparison) in jump thresholds during the 90 min interval following a 2oC CWS. A difference score analysis was performed because significant differences in vehicle threhsolds were observed among age groups.


Jump Threshold Difference Scores (mA)
-.04
0
.04
.08
.12
.16
30
60
90
Post-Swim (Min)
Cohort (Mo)
4
9
14
19
24

jump test declines with age. However, the effect does not appear to be as systematic as that indicated on the tail flick test.

Correlations between age and the combined analgesia score of the 30 and 60 min post-swim intervals at each water temperature were computed. A significant relationship was found following the 15°C ($r=-.633$; $r^2=.401$, $p<.01$) and 2°C ($r=-.513$; $r^2=.263$, $p<.01$) swim conditions. Thus, it appears that the degree of CWS analgesia on the jump test is inversly related to age for these two water temperatures. The tendency for lower jump threshold scores to be asssociated with older animals was only apparent at the intermediate and exteme levels of the water temperature range used.

CWS Hypothermia: Figure 12 and Tables 19 and 20 summarize the alterations in core body temperature following CWS across age groups. In the 4-month age group there were significant decreases in body temperature relative to the no swim condition 0 and 30 min following the 21°C swim, and then for up to 60 min following the 15, 8 and 2°C swim conditions. The 9, 14, and 19-month age groups showed similar patterns of hypothermia. Significant decreases in core body temperature were seen for up to 30 min following the 21 and 15 swims, for up to 60 min following the 8°C swim, and for the entire time course following the 2°C swim. The 24-month age group displayed significantly lower core body temperatures relative to the no swim conditions for up to 60 min following the 21 and 15°C swims, and for the entire time course following the 8 and 2°C swim conditions. Therefore, although all age groups showed a progressive increase in hypothermia with decreases in water temperature, the duration of the hypothermia was greater (longer)

Table 19: Alterations in core body temperature (°C) following cold water swim (CWS) across Age Groups.

Age Group	Swim Condition	Post-Injection (min)			
(°C)	Pre	0	30	60	90
4-month					
No Swim	38.16	38.15	38.23	38.31	38.25
	(.094)	(.087)	(.079)	(.119)	(.120)
21	38.44	35.21*	36.15*	37.89	38.02
	(.125)	(.232)	(.204)	(.154)	(.163)
15	38.51	34.23*	35.72*	37.12*	38.20
	(.199)	(.211)	(.258)	(.094)	(.158)
8	38.51	33.77*	33.47*	36.99*	38.35
	(.131)	(.388)	(.392)	(.265)	(.107)
2	38.42	31.44*	30.40*	34.81*	37.31
	(.118)	(.282)	(.817)	(.519)	(.247)
9-month					
No Swim	38.32	38.37	38.53	38.49	38.52
	(.156)	(.178)	(.154)	(.179)	(.156)
21	38.41	34.70*	35.69*	37.72	38.45
	(.222)	(.131)	(.202)	(.280)	(.196)
15	38.39	34.14*	35.19*	37.69	38.53
	(.237)	(.478)	(.475)	(.243)	(.246)
8	38.46	33.00*	33.29*	37.02*	38.41
	(.219)	(.258)	(.258)	(.027)	(.295)
2	38.45	30.54*	29.60*	33.94*	37.03*
	(.167)	(.028)	(.302)	(.183)	(.097)
14-month					
No Swim	38.06	38.12	38.01	37.99	38.21
	(.170)	(.155)	(.109)	(.120)	(.187)
21	38.50	35.60*	35.66*	37.85	38.15
	(.144)	(.268)	(.173)	(.226)	(.147)
15	38.22	35.26*	34.72*	37.27	37.94
	(.082)	(.547)	(.486)	(.330)	(.243)
8	37.75	33.81*	32.70*	36.00*	37.47
	(.238)	(.389)	(1.194)	(.752)	(.365)
2	38.22	31.99*	30.64*	31.97*	37.02*
	(.192)	(.376)	(.547)	(.520)	(.440)

Table continues on next page

(Table 19 cont.): Alterations in core body temperature (^{o}C) following cold-water swim (CWS) across age groups:

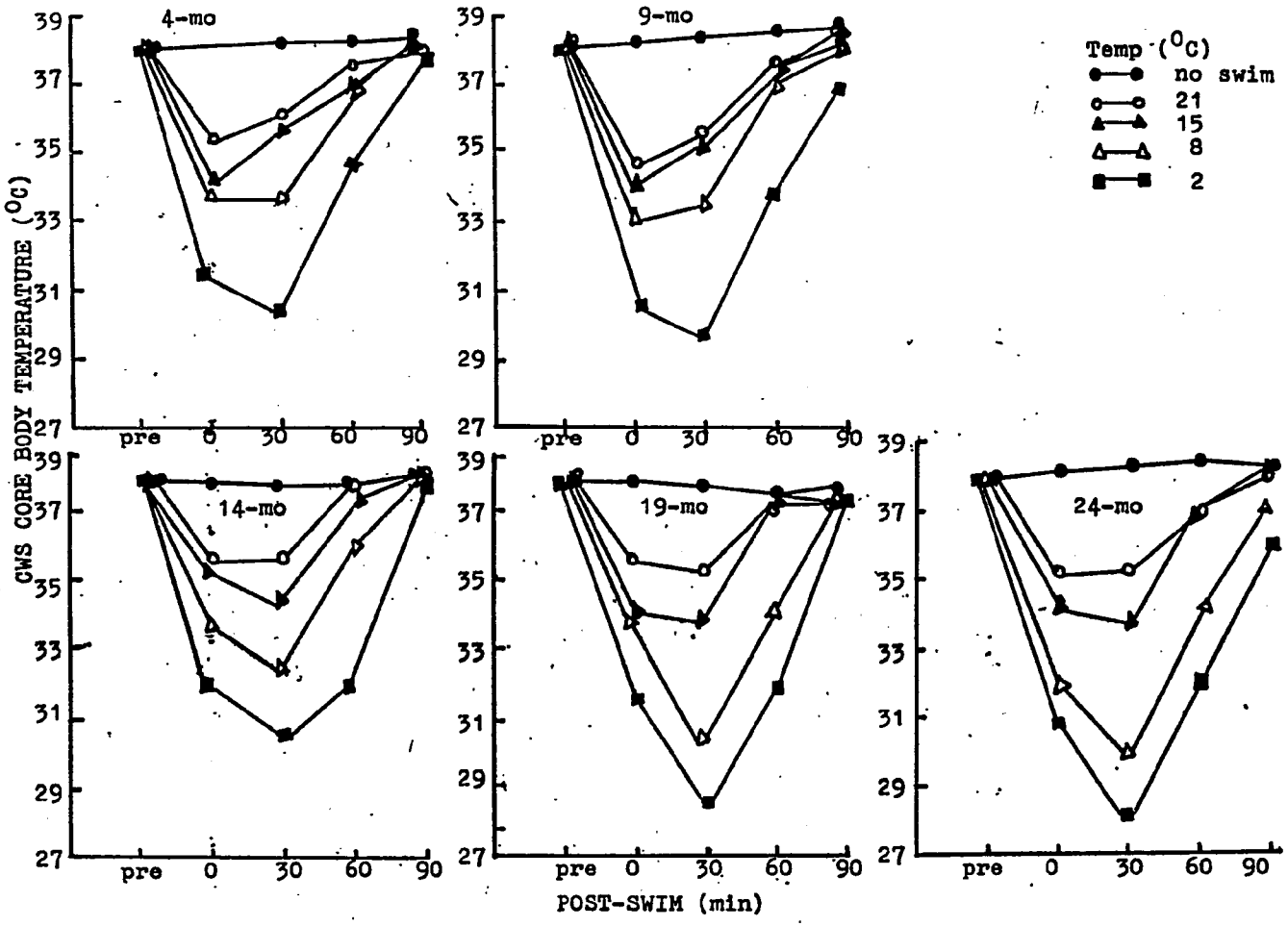
Age Group	Swim (^{o}C)	Pre	Post-Injection (min) 0	30	60	90
19-month						
	No Swim	38.39	38.39	38.42	38.41	38.39
		(.210)	(.198)	(.202)	(.208)	(.211)
	21	38.46	35.42*	35.14*	37.34*	38.06
		(.208)	(.307)	(.448)	(.276)	(.292)
	15	38.12	34.89*	34.42*	37.49	38.27
		(.237)	(.345)	(.396)	(.298)	(.282)
	8	38.36	33.91*	30.50*	34.14*	37.45
		(.312)	(.375)	(.961)	(1.230)	(.253)
	2	38.51	31.22*	28.34*	31.86*	36.92*
		(.276)	(.457)	(.894)	(.869)	(.695)
24-month						
	No Swim	38.30	38.35	38.43	38.65	38.53
		(.198)	(.178)	(.196)	(.167)	(.115)
	21	38.37	35.05*	35.25*	36.90*	38.22
		(.074)	(.258)	(.300)	(.321)	(.057)
	15	38.10	34.13*	33.73*	37.27*	38.15
		(.275)	(.253)	(.487)	(.283)	(.109)
	8	38.05	31.97*	30.03*	34.55*	37.38*
		(.146)	(.184)	(.604)	(.527)	(.163)
	2	38.37	30.88*	27.87*	32.12*	36.37*
		(.126)	(.484)	(.605)	(.529)	(.604)

Note: * significant difference ($p<.01$, Dunnett comparison) from corresponding vehicle value.

Table 20: Summary of the Analysis of Variance for Core Body Temperature Following CWS Across Age Groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	72.43	4	18.11	2.42	>.050
Swim Cond.(D)	2394.93	4	598.73	295.50	<.001
Time (T)	3062.37	4	765.59	584.12	<.001
A x D	79.68	16	4.98	2.46	<.010
A x T	101.97	16	6.37	4.86	<.001
D x T	1421.80	16	88.86	117.87	<.001
A x D x T	95.22	64	1.49	1.97	<.001

Figure 12. Alterations in core body temperature during the 90 min interval following CWS in different age groups.

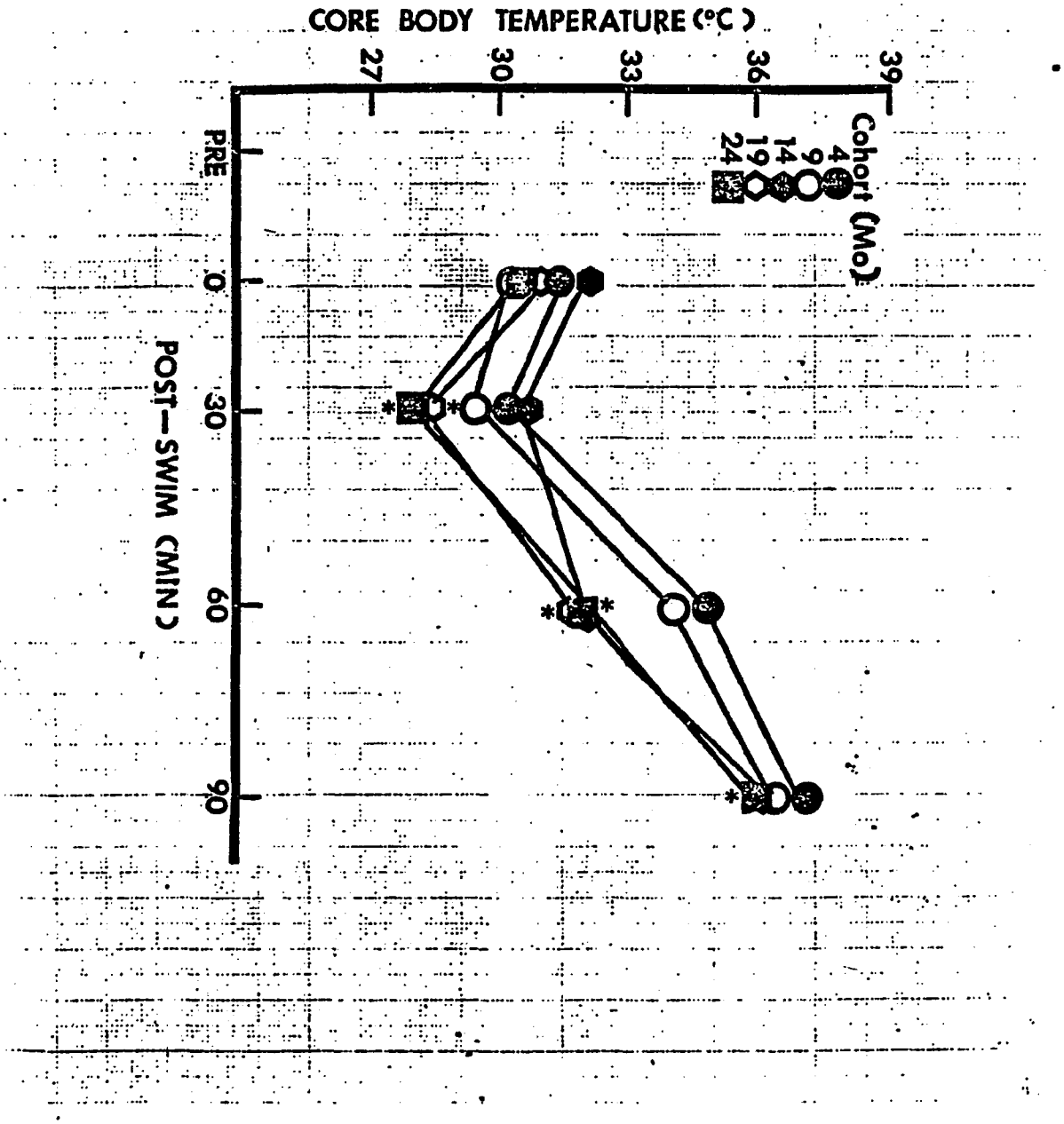

4-mo
9-mo
14-mo
19-mo
24-mo
Temp (°C)
no swim
21
15
8
2
CWS CORE BODY TEMPERATURE (°C)
POST-SWIM (min)
pre
0
30
60
90
27
29
31
33
35
37
39

in the older than in the younger animals.

Relative to the 4-month age group, no differences in core body temperature were observed across age groups in the no swim condition. Similaly, equivalent core body temperatures were found across all but the lowest water temperatures between the 4-month and older age groups, although the 19 and 24-month age groups displayed lower temperatures after a $8^{o}C$ swim. Figure 13 shows the changes in core body temperature during the 90 min time course following the $2^{o}C$ swim. Relative to the 4-month age group, no differences in core body temperature were found for the 9-month age group. However, the 14, 19 and 24-month age groups demonstrated significantly greater hypothermic responses than the 4 month old group.

To examine whether increases in CWS hypothermia were linearly related to age, the body temperatures following the several swim conditions were correlated with age. Significant negative correlations between age and core body temperature were obtained following the $21^{o}C$ ($r=-.459$; $r^2=.211$, $p<.01$), $15^{o}C$ ($r=-.337$; $r^2=.114$, $p<.05$), $8^{o}C$ ($r=-.548$; $r^2=.300$, $p<.01$) and $2^{o}C$ ($r=-.486$; $r^2=.236$; $p<.01$) swim conditions. Thus, with increase in age the hypothermic effect of CWS increased in degree and duration.

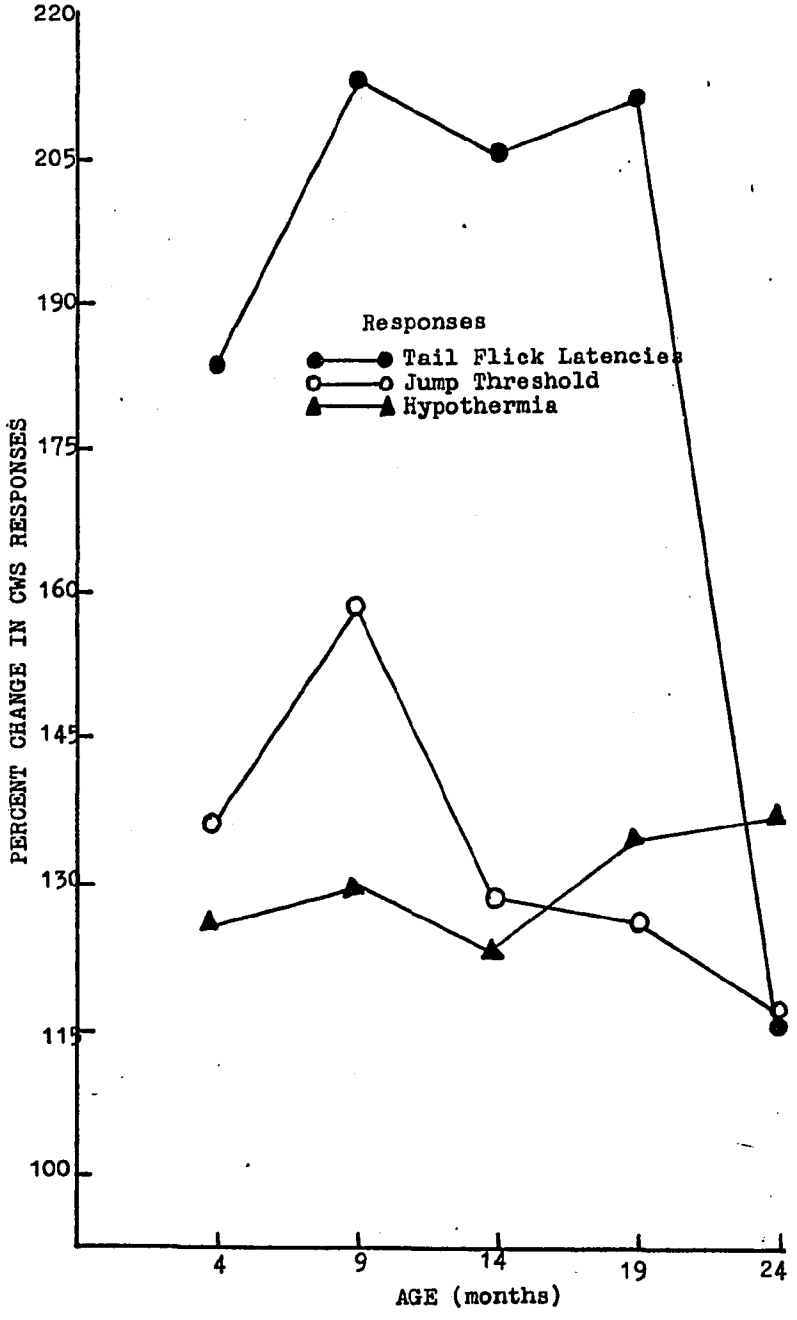
Figure 13. Alterations (*Dunnet comparison) in core body temperature during the 90 min interval following a 2oC CWS in different age groups.


CORE BODY TEMPERATURE (°C)
27
30
33
36
39
Cohort (Mo)
4
9
14
19
24
PRE
0
30
60
90
POST—SWIM (MIN)

Discussion

Significant decreases in CWS analgesia and significant increases in CWS hypothermia occurred with increases in age. The age-related analgesic decrements occurred abruptly, in that only the oldest age group exhibited significant decreases in latency on the tail flick test. Figure 14 compares the changes in response patterns observed across cohorts for tail flick latencies, jump thresholds and core body temperature. The percentage change in tail flick latencies 30 min following the 2°C CWS was 186% in the 4-month group, 216% in the 9-month group, 207-212% in the 14 and 19-month groups, and only 116% in the 24-month group. The age-related decrement was more gradual in the jump test, with lower analgesic jump thresholds observed for the 14, 19, and 24-month groups than for the younger groups. The decrease in CWS analgesia with increases in age on both tests occurred independently of age-related changes in baseline pain thresholds since baseline tail-flick latencies failed to differ among age groups, and the small but significant hyperresponsivenss noted for the 24-month group on the jump test was factored out of the difference-score analysis. Although baseline core body temperatures were similar across age groups, increased hypothermic responses were found following CWS with increases in age. This age-related change was also gradual, with significant age differences first apparent at 14 months, and most pronounced at 24 months of age. The reduction in CWS analgesia and the potentiation of CWS hypothermia with increase in age indicate altered adaptive responses to CWS stress, and

Figure 14. Percent change in tail-flick latency, jump threshold and food intake responses 60 min following a 650 mg/kg 2DG dose in different age groups.


220
205
190
175
160
145
130
115
100
PERCENT CHANGE IN CWS RESPONSES
Responses
Tail Flick Latencies
Jump Threshold
Hypothermia
4
9
14
19
24
AGE (months)

suggest that the two CWS responses are mediated through different mechanisms of action.

The present results agree with reports of a delayed and less complete adaptation to the opiate-sensitive analgesia induced by intermittent CWS in older (15-16 mo) as compared to younger (4 mo) rats (Giradot and Holloway, 1984). Intermittent cold water swim (ICWS) analgesia is said to be under opioid control, as it is antagonized by the opiate receptor antagonist naltrexone, and cross tolerant with morphine analgesia. In addition to the reduced abiilty to show adaptation after repeated exposure to ICWS, it has been found that older rats display reduced cross tolerance to morphine. That is, although young rats exposed to chronic, opiate-sensitive, intermittent CWS displayed decrements in subsequent morphine analgesia, older rats exposed to the same regimen failed to display such effects (Giradot and Holloway, 1985).

The present findings do not agree with a report by Hamm and co-workers (1985) who found age-dependent increases in CWS analgesia. There were several procedural differences between the two studies, however. Hamm and colleagues used two groups of male rats of significantly different weights: 3-month (343g) and 23-month (809g). In contrast, the present study employed five groups of female rats with more evenly distributed weights: 4-month (274.6), 9-month (291.2), 14-month (353.8), 19-month (326.4), and 24-month (311.4) were used. Indeed, the confounding of gender and weight in developmental studies such as that of Hamm et al., was a primary reason for the use of female rats in our experiment.

Therefore, the opposite results obtained by the two studies might be explained in terms of gender or weight differences. Whereas systematic analyses of gender differences in CWS analgesia have not been undertaken, the magnitude of CWS analgesia has been found to be positively correlated with body weight (Bodnar, Kelly, Glusman, 1978). Moreover, in a study in which the effects of cold exposure on core body temperature and neurotransmitter release in male rats as a function of age were examined (Algeri et al, 1982), the thickness of subcutaneous adipose tissue levels were found to affect sensitivity to cold, and so older rats (18, 29 mo) were placed on a restricted diet before testing. Therefore, the reported increase in CWS analgesia in older and heavier male animals might be attributed to weight and not age.

There are other differences between the present study and that of Hamm and colleagues (1985). Whereas the present study found progressive, age-dependent increases in CWS hypothermia at 30-120 min following the swims, Hamm and colleagues (1985) found similar hypothermia between the two age groups at their only data point measured immediately after the swim. It should be noted that peak CWS hypothermia occurs between 15 and 30 min following the swim (Bodnar et al., 1978, 1979), and might be more sensitive to age-dependent adaptive effects. In any case, future work is needed to ascertain the relevant variables which can account for the different results.

The mediation of CWS analgesia has been attributed to both opioid and nonopioid processes. A role for opioid processes has been suggested by the findings that CWS analgesia is partially

reversed by nalaxone under some conditions (Bodnar and Sikorszky, 1983; Giradot and Holloway, 1984a). This ability to be reversed by nalaxone appears to be dependent on such variables as swim duration, water temperature, post-swim interval and magnitude of analgesic response induced in individual animals. Further, like morphine, CWS analgesia is potentiated in rats with either adrenelectomy, neuroleptic pretreatment, or destruction of the medial-basal hypothalamus (Glusman, et al, 1980; Holoday et al., 1979; Bodnar, Abrams, et al., 1980). Again, the reduced analgesic responsivity of the aged rat may be related to lower opioid levels and decreased opiate receptor binding (Jensen et al., 1980; Gambert et al., 1980). On the other hand, it is clear that CWS analgesia is not completely modulated by an opioid mediated system since CWS analgesia is unaffected by morphine tolerance and nalaxone (Bodnar et al., 1979, 1983). Moreover, the high affinity opiate antagonist, naloxazone potentiates the analgesiac effect of cold water swim (Kirchgessner et al., 1982), and the anti-enkephalinase, D-phenylalanine reduces its analgesic effect (Bodnar et al, 1980).

In addition to opioid system modulation of CWS analgesia, Hypothalamo- hypophyseal-adrenal modulation has been implicated in view of the finding that CWS activates the pituitary-adrenal axis and increases blood levels of ACTH and corticosterone (Seyle, 1952). Moreover, CWS analgesia is reduced in either hypophysectomized (anterior and posterior pituitary gland), vasopressin- deficient (posterior pituitary gland) or dexmethasone (anterior pituitary gland) treated rats (Bodnar et al., 1979; Mousa et al, 1983; Marek et al., 1982). Age-dependent alterations in

hypothalamo- hypophyseal interactions have also been reported in the functioning of the anterior and posterior lobes of the pituitary gland, including reductions in levels of hypothalamic vasopressin (Zbuzek and Wu, 1982), glucocorticoid receptor binding (Roth, 1976), and sensitivity of the hypothalamic-hypophyseal axis to feedback inhibition by glucocorticoids (Dilman et al., 1979). Moreover, as these impairments are aggravated in response to stress (Brett et al, 1983; Tang and Phillips, 1978; Hess and Riegle, 1970), they may explain the declines in analgesic responsivity observed with increase in age.

Catecholamines are also involved in CWS analgesia. Following CWS, brain stores of norepinephrine are released and subsequently depleted (Stone, 1970; Ritter et al., 1978). Further, pretreatment with both the norepinephrine reuptake inhibitor, desipramine, and the noradrenergic receptor stimulant, clonidine, potentiate CWS analgesia (Bodnar, Mann and Stone, 1985; Bodnar, Merrigan and Sperber, 1983). In this regard, senescent rodents display greater reductions in norepinephrine and serotonin levels following cold exposure than do younger animals. Moreover, there is an imbalance between dopaminergic and serotonergic systems so that whereas cold exposure results in an increase in dopamine turnover and tyrosine hydroxylase activity with no change in sertononin turnover, the opposite occurs in aged rats (Algeri et al., 1982; Brady et al, 1980; Ritter and Pelzer, 1978; Thurmond and Heishman, 1984).

Systematic progressive increases in CWS hypothermia as a function of age were also found in the present study. Thus, although adaptive thermal and vasoconstrictive responses to

exposure to the cold were manifested by all animals, they are altered during the aging process. The age-related increases in CWS hypothermia and reductions in CWS analgesia found in our study are in agreement with prior observations of a dissociation between the two responses. CWS analgesia, but not CWS hypothermia, is decreased following hypophysectomy, D-phenylalanine pretreatment and repeated exposure to CWS (Bodnar et al, 1978, 1979, 1980). Moreover, acute desiprimine pretreatment increases CWS analgesia, but not CWS hypothermia (Bodnar et al., 1985). However, other manipulations alter both responses similarly. CWS analgesia and CWS hypothermia are increased by clonidine pretreatment and decreased by neonatal MSG treatment (Badillo-Martinez et al.,1984, Bodnar et al, 1983). The latter two effects suggest that analgesic magnitude can be affected by hypothermic magnitude; this is supported by decreases in CWS analgesia observed as water temperature increases (Bodnar et al., 1979; Bodnar and Sikorsyky, 1983). Therefore, this would imply that the age-related declines in CWS analgesia should be accompanied by similar decreases in CWS hypothermia in the older age groups. However, in the present study, the opposite results were obtained. CWS hypothermia was more pronounced following the 2^{o}C swim at 60 min in the 14-mo group, at 30 and 60 min in the 19-month group and across the entire 120 min time course in the 24-month group; this suggests that CWS hypothermia and analgesia reflect two independent adaptive responses to stress.

The current finding of longer persistence of CWS hypothermia with increase in age suggests that thermoregulatory recovery is

impaired with increase in age, and agrees with reports of less efficient thermoregulatory control in older rats as reflected in greater reductions in core body temperature and longer recovery times following exposure to a cold environment or cold water solutions (Segal and Timiras, 1975; Thurmond and Heishman, 1984; Hamm, 1981; McDougal et al, 1981). The reduced ability of older animals to thermoregulate has been associated with impaired peripheral functioning involving gluconeogenesis and adrenal responsivity (Finch, Foster and Mirsky, 1965). Central factors have also been implicated, including reduced responsiveness of the catecholaminergic systems, smaller increases in plasma corticosterone levels, greater reductions in norephinephine and seretonin levels, and an imbalance between dopamine and serotonin systems (Thurmond and Heishman, 1984; Algeri et al., 1982; Brady et al., 1980). Whereas no study has found an association between the decrements in CWS analgesia and the above mentioned variables, the present results suggest that the decrements in CWS analgesia in older animals are the result of changes in either other stress-related responses (Pfeifer and Davis, 1975; McCarty, 1981, Guttman, 1970; Frolkis, 1972) or in endogenous pain-inhibitory systems (see reviews: Bodnar, 1984). The lack of CWS analgesia also appears to be due to changes in the stressful consequences of the swim, rather than general changes in reactivity to swim per se because the hypothermic response was present.

Experiment 3: Morphine

Method

Morphine analgesia and thermoregulation were assessed for 42 rats in the following 5 age groupss: 4 (n=10), 9 (n=10), 14 (n=8), 19 (n=8), and 24 (n=6) months of age. Five doses of morphine were administered in ascending order, with a minimum of 4 days elapsing between each injection so as to avoid tolerance effects. The dosages, established in previous studies (Bodnar, Kelly, Mansour and Glusman, 1979) were: 0, 1.0, 2.5, 5.0 and 10.0 (mg/ml saline/kg body weigh, SC). Tail flick latencies and jump thresholds were assessed at 30, 60, 90 and 180 minutes following each injection. Core body temperatures were obtained from each rat immediately prior to injection as well as at 30, 60, 90 and 180 min post-injection intervals.

Results

Tables 21 through 28 show the mean absolute and mean difference mean scores, standard error of the means, and significant main effects and interaction for tail-flick latencies, jump thresholds, and core body temperature following morphine administration. Figures 15 through 21 depict the effects of morphine as a function of age at selected post-injection intervals and doses.

Morphine Analgesia (Tail-Flick Test): Figure 15 and Tables 21 and 22 summarize the alterations in morphine analgesia on the

Table 21. Alterations in tail-flick latencies (sec:SEM) following morphine across Age Groups.

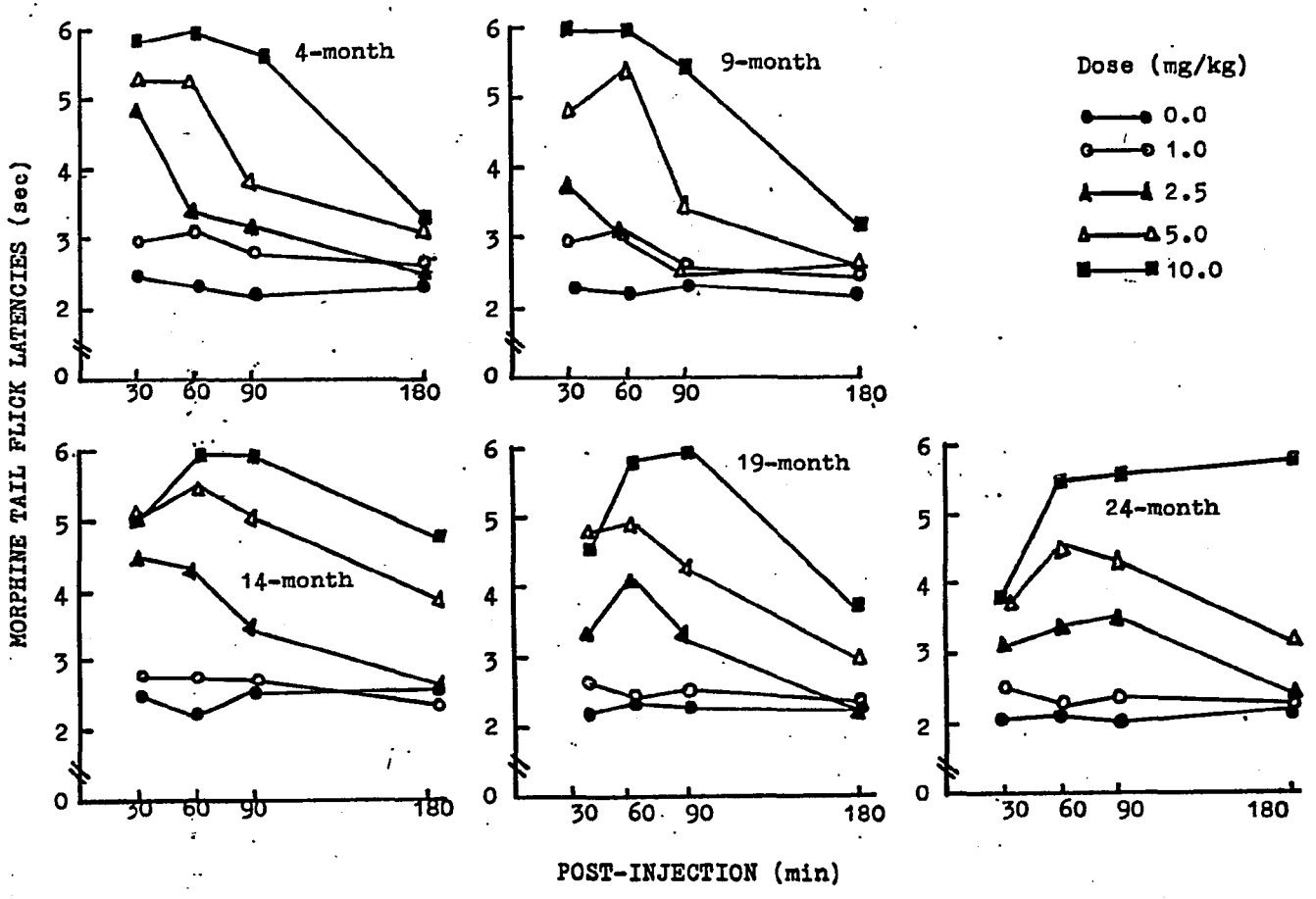
Age Group / Morphine Dose (mg/kg)	Post-Injection (min) 30		60		90		180	
4-month	mean	SEM	mean	SEM	mean	SEM	mean	SEM
0	2.41	(.079)	2.32	(.116)	2.19	(.068)	2.31	(.069)
1.0	3.04	(.183)*	3.09	(.141)*	2.86	(.154)	2.71	(.087)
2.5	4.91	(.285)*	3.44	(.219)*	3.19	(.149)*	2.58	(.169)
5.0	5.35	(.234)*	5.33	(.139)*	3.86	(.288)*	3.13	(.259)*
10.0	5.90	(.070)*	6.00	(.000)*	5.68	(.131)*	3.27	(.327)*
9-month								
0	2.29	(.051)	2.19	(.086)	2.23	(.078)	2.09	(.045)
1.0	2.98	(.150)	3.02	(.182)*	2.62	(.120)	2.45	(.097)
2.5	3.69	(.308)*	2.94	(.236)	2.59	(.108)	2.66	(.274)
5.0	4.85	(.333)*	5.55	(.200)*	3.46	(.261)*	2.54	(.209)
10.0	5.96	(.025)*	6.00	(.000)*	5.44	(.308)*	3.09	(.200)*
14-month								
0	2.52	(.087)	2.34	(.105)	2.50	(.082)	2.52	(.081)
1.0	2.77	(.175)	2.75	(.214)	2.67	(.195)	2.39	(.048)
2.5	4.48	(.410)*	4.32	(.477)*	3.53	(.455)*	2.77	(.169)
5.0	5.01	(.412)*	5.63	(.288)*	5.07	(.506)*	3.88	(.289)*
10.0	4.98	(.384)*	6.00	(.000)*	5.93	(.065)*	4.65	(.487)*
19-month								
0	2.22	(.101)	2.30	(.114)	2.29	(.054)	2.16	(.052)
1.0	2.65	(.137)	2.44	(.119)	2.48	(.125)	2.32	(.077)
2.5	3.19	(.440)*	4.07	(.291)*	3.38	(.410)*	2.21	(.042)
5.0	4.77	(.517)*	4.97	(.462)*	4.17	(.494)*	3.03	(.233)*
10.0	4.39	(.520)*	5.76	(.244)*	6.00	(.000)*	3.67	(.508)*
24-month								
0	2.08	(.112)	2.12	(.081)	2.04	(.060)	2.18	(.121)
1.0	2.50	(.228)	2.40	(.063)	2.35	(.162)	2.32	(.148)
2.5	3.09	(.205)*	3.42	(.538)*	3.60	(.731)*	2.40	(.145)
5.0	3.62	(.530)*	4.53	(.450)*	4.40	(.732)*	3.26	(.496)*
10.0	3.87	(.627)*	5.56	(.443)*	5.60	(.403)*	5.81	(.164)*

Note: * Significant difference ($p<.05$, Dunnett comparison) from corresponding vehicle value.

Table 22: Summary of the Analysis of Variance for Tail Flick Latencies following Morphine across Age Groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	26.17	4	6.54	2.48	>.050
Dose (D)	936.02	4	234.00	353.74	<.001
Time (T)	125.06	3	41.69	83.88	<.001
A x D	16.38	16	1.02	1.55	>.050
A x T	44.21	12	3.69	7.41	<.001
D x T	92.33	12	7.69	19.62	<.001
A x D x T	68.03	48	1.42	3.61	<.001

Figure 15. Alterations in tail-flick latencies during the 180 min interval following morphine in different age groups.


4-month
9-month
14-month
19-month
24-month
Dose (mg/kg)
0.0
1.0
2.5
5.0
10.0
MORPHINE TAIL FLICK LATENCIES (sec)
POST-INJECTION (min)
0
2
3
4
5
6
30
60
90
180

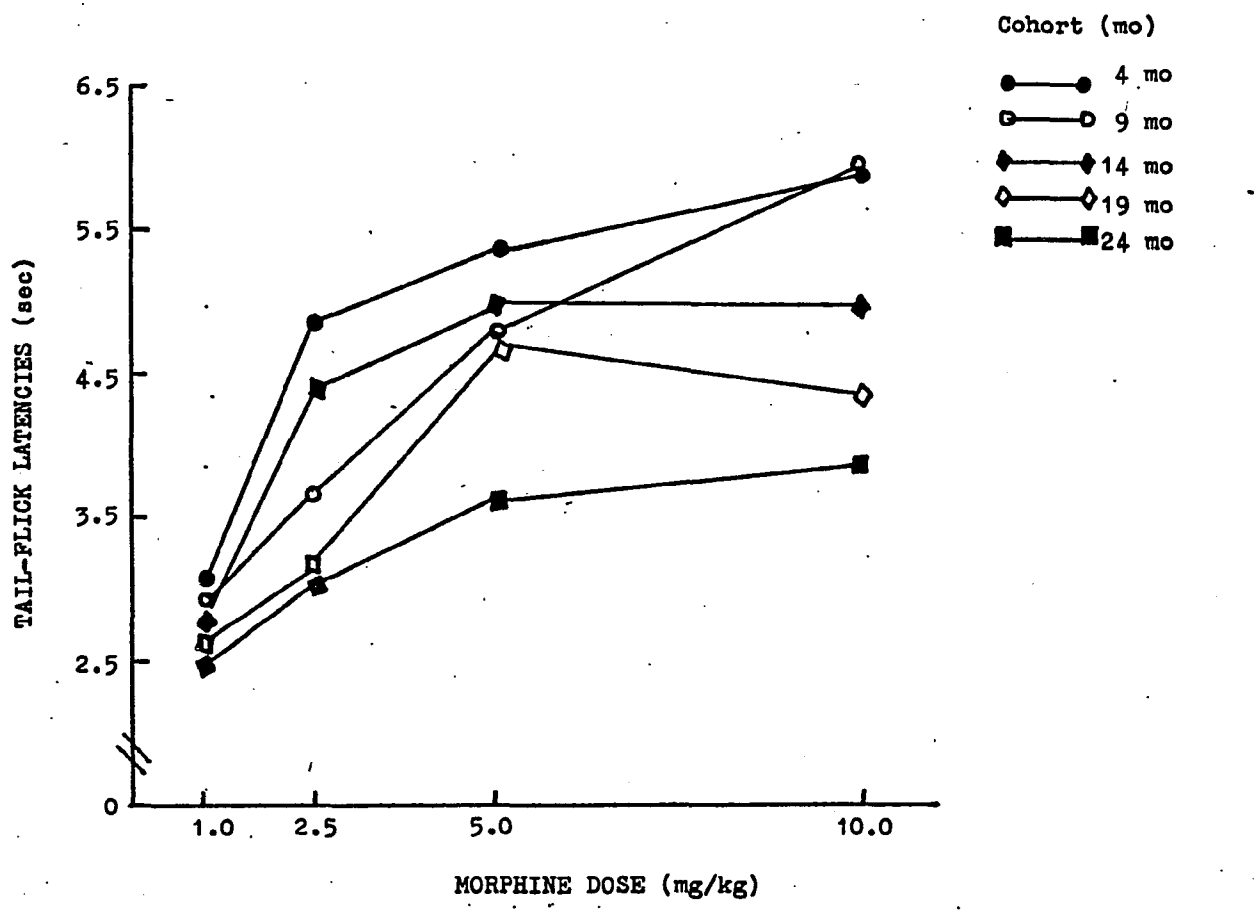
tail-flick test across age groups. The pattern of latency responses were generally similar for the 4 and 9-month age groups on the one hand, and for the 14-24 month age groups on the other hand. The 4 and 9-month age groups displayed a systematic dose-related increase in morphine analgesia that was significant 30 min following the 1.0 mg/kg dose for the 4-month group, for up to 90 (4-month) and 30 (9-month) min following the 2.5 mg/kg dose, for up to 180 (4-month) and 90 (9-month) min following the 5.0 mg/kg dose, and across the 180 min time course of both groups following the 10.0 mg/kg dose. The three older age groups displayed similar patterns of analgesic responsivity, with no increases in tail-flick scores following the 1.0 mg/kg dose, and signficiant increases in tail-flick latencies up to 90 min following the 2.5 mg/kg dose, and for up to 180 min following the 5.0 and 10.0 mg/kg doses. Therefore, although the duration of morphine analgesia on the tail flick test increased in all age groups with increase in dose levels, a gradual decline in tail flick scores was found after 60 min in the 4 and 9-month age groups, and only after 90 min in the three older age groups. Moreover, the degree of analgesia on the tail flick test varied as a function of morphine dose administered. Whereas the 4 and 9-month age groups displayed a progressive systematic increase in tail flick latencies with increasing morphine dose, the peak analgesic effect was reached at the 5.0 dose level and then maintained for the 10.0 mg/kg dose level in the 14, 19 and 24-month age groups.

No differences in tail flick latencies were observed across age groups following the vehicle injection. Figure 16 summarizes

the differences among age groups relative to the 4-month age group at 30 min following the various doses of morphine. All age groups showed shorter tail flick latencies 30 min following the 2.5 mg/kg dose, but only the 19 and 24-month age groups showed significantly shorter latencies at the highest and two highest morphine doses, respectively. In contrast, at the later post-injection intervals, the older age groups signficantly increased tail flick latencies relative to the 4-month age group: at the 60 min interval following the 2.5 and 5.0 mg/kg dose (14 mo) and at the 180 min post-injection interval following the 10.0 mg/kg dose (14 and 24 mo). Therefore, with increase in age from 14-24 mo, there was a tendency for reduced analgesia on the tail flick test at the early post-injection interval, but once elicited the analgesia persisted for a longer period of time relative to the younger age groups.

Age-related changes in morphine analgesia were examined by correlating the latency score at 30 and 180 post-injection intervals with age. These two intervals were selected because they appeared to represent the period where age differences were most evidenced. Statistically significant negative correlations between age and analgesic score were found 30 min following the 2.5 ($r=-.472$; $r^2=.223$, $p<.01$), 5.0 ($r=-.367$; $r^2=.135$, $p<.05$), and 10.0 ($r=-.628$; $r^2=.394$, $p<.01$) mg/kg doses, and 180 min following the 10.0 mg/kg dose ($r=.529$; $r^2=.280$, $p<.01$). Therefore, it appears that the relationship between age and tail flick latency following morphine administration varies as a function of the post-injection interval. At the 30 min test interval, there was a tendency for older age groups to display longer tail flick latencies than

Figure 16. Alterations (*Dunnett comparison) tail-flick latencies at 30 min following morphine in different age groups.


Cohort (mo)
4 mo
9 mo
14 mo
19 mo
24 mo
TAIL-FLICK LATENCIES (sec)
6.5
5.5
4.5
3.5
2.5
0
1.0
2.5
5.0
10.0
MORPHINE DOSE (mg/kg)

younger age groups; this relationship was dose dependent. However, at 180 min following morphine injections, there was a tendency with increases in age for higher tail flick latency scores; this relationship was significant only at the highest dose.

Morphine Analgesia (Jump Test):. Figure 17 and Tables 23 and 24 summarize the alterations in jump thresholds following morphine administration across age groups. Although none of the cohorts displayed increases in jump thresholds following the 1.0 mg/kg dose of morphine, age differences were found following the higher doses. The 4 and 9-month age groups displayed systematic dose related increases in analgesia for up to 60 min following the 2.5 mg/kg dose, and for up to 90 min (4-month) and 180 min (9-month) following the 5.0 and 10.0 mg/kg doses. In contrast, increases in jump thresholds were found only at the 10.0 mg/kg dose (up to 60 min) for the 14-month age group, at the 2.5 and 5.0 mg/kg (up to 30 min) and 10.0 mg/kg dose (up to 60 min) for the 19-month age group, and for up to 90 min following the 2.5, 5.0, and 10.0 mg/kg doses for the 24-month age group. Therefore, the degree and duration of analgesia on the jump test across age groups was dependent on the dose and the interval. However, whereas the 4 and 9-month age groups showed a progressive increase in jump threshold that was more persistent with increases in doses, the 14, 19 and 24-month age groups generally showed equivalent jump threshold scores across doses and post-injection intervals.

Since jump thresholds following the vehicle injection differed significantly among age groups, with the 19 and 24-month groups displaying hyperresponsiveness, a difference score analysis

Table 23. Alterations in jump thresholds (mA: SEM) following morphine across age groups.

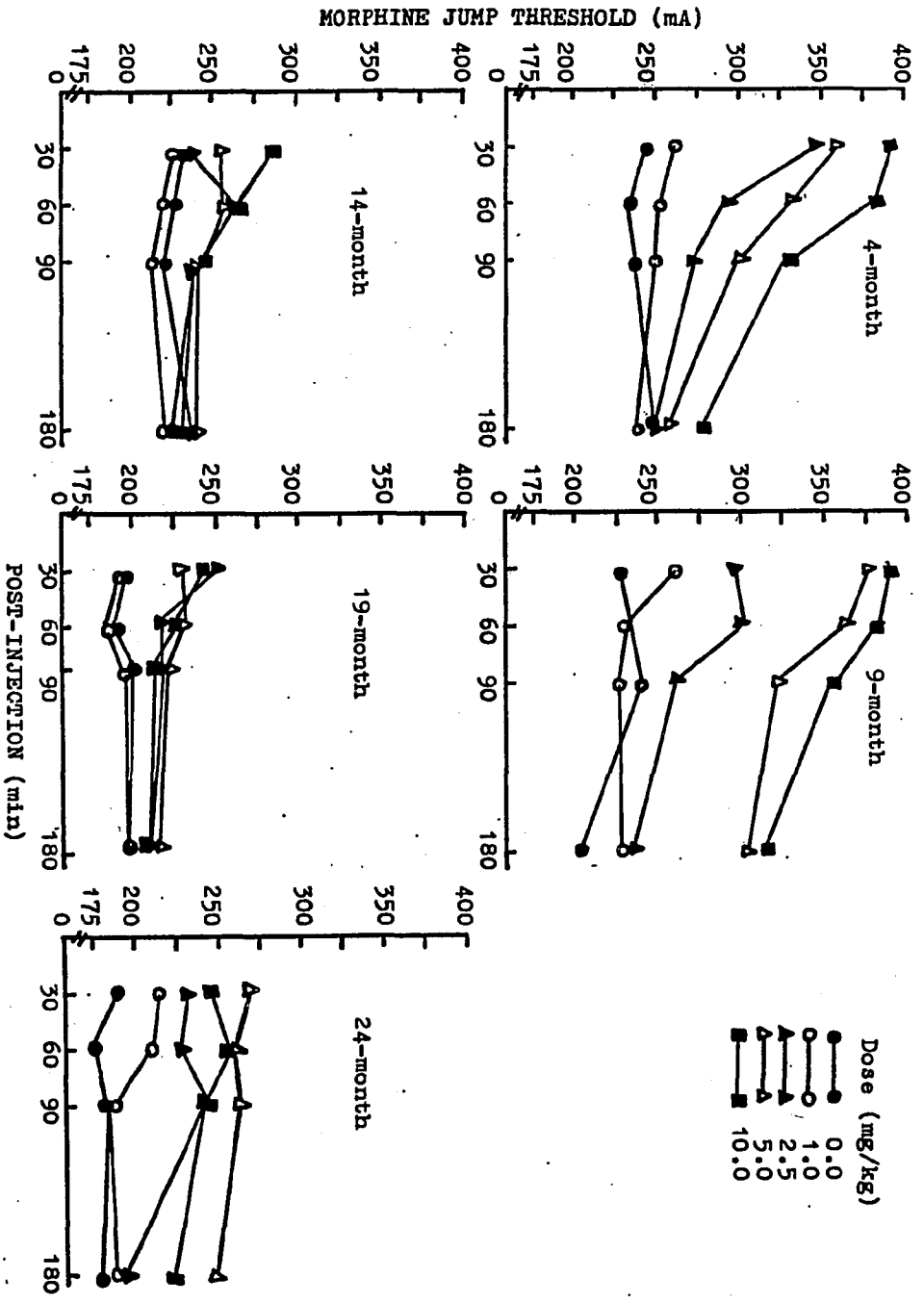
Age Group / Morphine Dose (mg/kg)	Post-Injection (min) 30		60		90		180	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
4-month								
0	.243	(.008)	.233	(.007)	.238	(.008)	.245	(.009)
1.0	.263	(.008)	.252	(.010)	.250	(.009)	.234	(.008)
2.5	.351	(.013)*	.294	(.009)*	.274	(.013)	.252	(.008)
5.0	.361	(.016)*	.337	(.014)*	.303	(.010)*	.262	(.012)
10.0	.393	(.014)*	.382	(.023)*	.327	(.023)*	.278	(.013)
9-month								
0	.228	(.015)	.232	(.020)	.237	(.018)	.208	(.018)
1.0	.263	(.008)	.231	(.008)	.228	(.010)	.224	(.007)
2.5	.295	(.007)*	.299	(.019)*	.265	(.025)	.231	(.018)
5.0	.375	(.024)*	.362	(.021)*	.320	(.017)*	.301	(.009)*
10.0	.386	(.016)*	.381	(.014)*	.355	(.011)*	.314	(.026)*
14-month								
0	.232	(.011)	.225	(.014)	.223	(.011)	.231	(.008)
1.0	.226	(.009)	.222	(.011)	.215	(.010)	.220	(.012)
2.5	.241	(.010)	.262	(.005)	.241	(.012)	.233	(.012)
5.0	.255	(.008)	.261	(.010)	.243	(.012)	.237	(.009)
10.0	.287	(.021)*	.266	(.008)*	.239	(.005)	.220	(.012)
19-month								
0	.195	(.013)	.190	(.019)	.204	(.015)	.203	(.017)
1.0	.192	(.012)	.183	(.015)	.197	(.014)	.202	(.015)
2.5	.253	(.017)*	.221	(.027)	.222	(.018)	.215	(.018)
5.0	.231	(.021)	.234	(.031)*	.222	(.024)	.214	(.021)
10.0	.240	(.028)*	.228	(.023)*	.218	(.022)	.220	(.014)
24-month								
0	.189	(.012)	.178	(.016)	.183	(.009)	.186	(.010)
1.0	.217	(.018)	.211	(.022)	.187	(.011)	.179	(.008)
2.5	.232	(.025)*	.228	(.015)*	.247	(.020)*	.192	(.022)
5.0	.271	(.016)*	.261	(.011)*	.264	(.011)*	.250	(.006)*
10.0	.244	(.024)*	.260	(.017)*	.239	(.008)*	.222	(.015)*

Note: * significant difference ($p<.05$), Dunnett comparison) from corresponding vehicle value.

Table 24: Summary of the Analysis of Variance for Jump Threshold Following Morphine across age groups.

Condition	SS	df	MS	F-value	p
Age Group (A)	0.83	4	0.20	12.12	<.001
Dose (D)	0.64	4	0.16	69.10	<.001
Time (T)	0.14	3	0.04	42.28	<.001
A x D	0.24	16	0.01	6.37	<.001
A x T	0.06	12	0.00	4.81	<.001
D x T	0.06	12	0.00	5.64	<.010
A x D x T	0.06	48	0.00	1.37	>.050

Figure 17. Alterations in jump thresholds during the 180 min interval following morphine in different age groups.


MORPHINE JUMP THRESHOLD (mA)
4-month
9-month
14-month
19-month
24-month
POST-INJECTION (min)
Dose (mg/kg)
0.0
1.0
2.5
5.0
10.0

of variance was performed to ascertain whether the magnitude of morphine analgesia differed among age groups. Figure 18 and Tables 25 and 26 summarize the differences among age groups relative to the 4-month age group. No differences in analgesia across age groups was found following the 1.0 mg/kg dose. However, the 9-month age group showed less morphine analgesia following the 2.5 mg/kg dose, and greater analgesia 180 min following the 10 mg/kg dose than did the 4-month age group. In contrast, the 14, 19 and 24-month age groups displayed progressive reductions in morphine analgesia relative to the 4-month age groups as doses increased. Less analgesia was observed 30 min following the 2.5 mg/kg dose in all groups, for up to 30 min (24-month), 60 min (19-month) and 90 (14-month) min following the 5.0 mg/kg dose, and for up to 60 (24-month), 90 (19-month) and 180 (14-month) min following the 10 mg/kg dose. Therefore morphine analgesia on the jump test was reduced in animals from 14 to 24 months of age as compared to the younger age groups. However, both the degree and duration of analgesia for animals of the 14-24 age range differed: the 24-month old rats showed higher jump thresholds that lasted longer than was the case for the 14 and 19-month age groups. These results indicate that while morphine analgesia is significantly reduced in the 14-month old relative to the 4-month age group, there may be the beginnings of a resurgence of the response in the 24-month group.

To examine the relationship between age and jump thresholds following morphine administration, correlations were computed between age and analgesia difference scores at the 30 min interval for each morphine dose. The 30 min interval was selected because

Table 25. Alterations in jump threshold difference scores (mA: SEM) as a function of age relative to the 4 month age group following morphine.

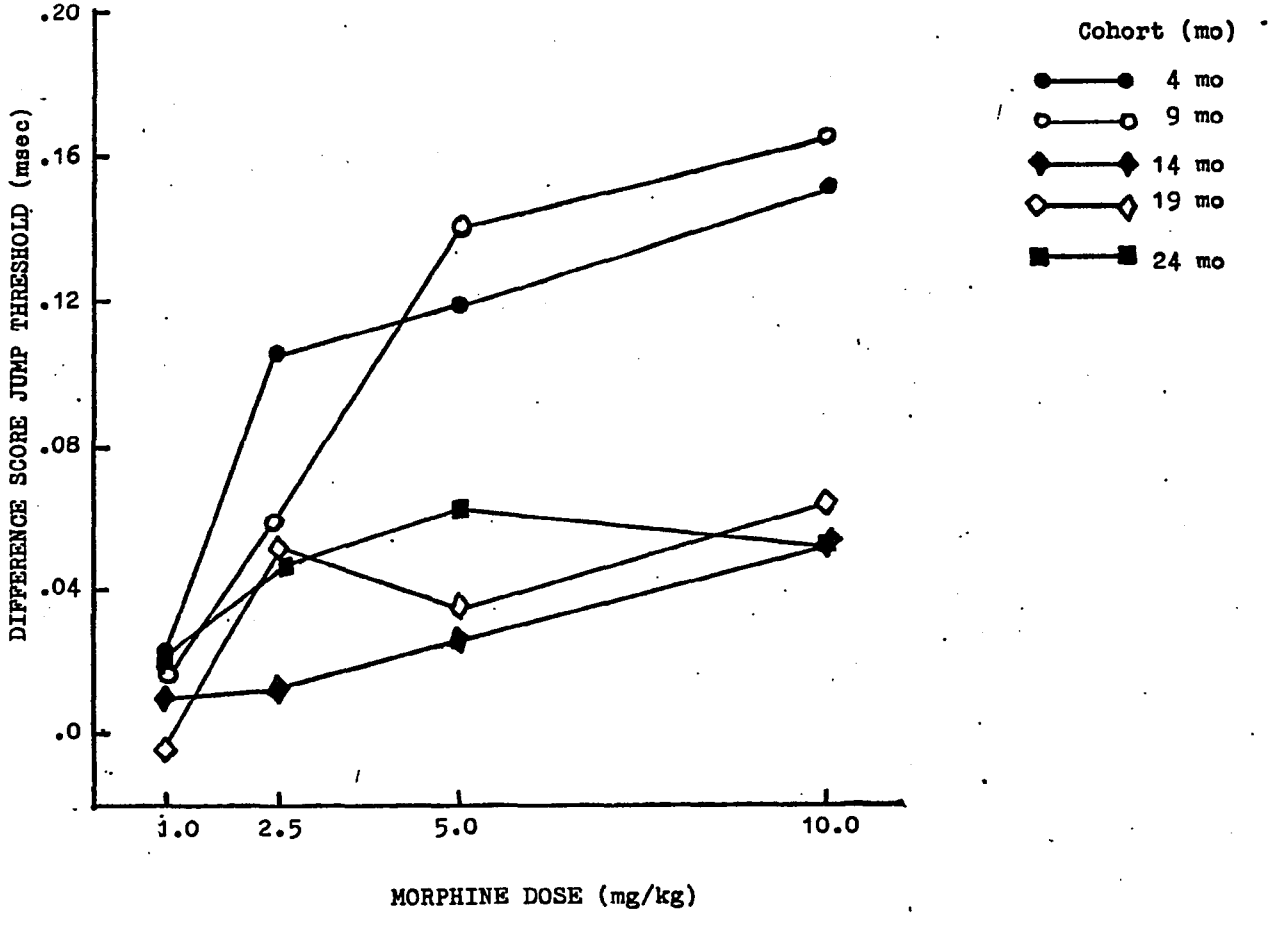
Age Group / Morphine Dose (mg/kg)	Post-Injection (min) 30		60		90		180	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
4-month								
1.0	.020	(.009)	.018	(.008)	.011	(.009)	-.011	(.010)
2.5	.108	(.012)	.061	(.011)	.036	(.012)	.010	(.010)
5.0	.110	(.014)	.103	(.017)	.065	(.011)	.018	(.014)
10.0	.151	(.016)	.138	(.020)	.088	(.028)	.034	(.017)
9-month								
1.0	.018	(.011)	-.001	(.018)	-.012	(.013)	.008	(.015)
2.5	.061	(.012)**	.071	(.016)	.033	(.022)	.018	(.017)
5.0	.140	(.023)	.138	(.017)	.077	(.021)	.082	(.020)
10.0	.163	(.018)	.152	(.024)	.115	(.022)	.102	(.029)*
14-month								
1.0	.006	(.010)	.003	(.019)	.008	(.007)	.011	(.008)
2.5	.007	(.011)**	.037	(.012)	.017	(.016)	.002	(.011)
5.0	.023	(.014)**	.036	(.016)**	.002	(.013)**	.007	(.008)
10.0	.054	(.026)**	.033	(.012)**	.013	(.011)**	-.012	(.008)
19-month								
1.0	-.003	(.010)	-.007	(.009)	-.007	(.013)	-.001	(.005)
2.5	.058	(.009)**	.030	(.012)	.019	(.015)	.006	(.009)
5.0	.036	(.013)**	.044	(.018)**	.011	(.006)**	.010	(.011)
10.0	.052	(.017)**	.023	(.016)**	.013	(.013)**	.016	(.013)
24-month								
1.0	.028	(.023)	.033	(.023)	.004	(.015)	.012	(.011)
2.5	.044	(.029)**	.050	(.023)	.064	(.014)	.006	(.014)
5.0	.065	(.015)**	.083	(.014)	.081	(.008)	.077	(.022)*
10.0	.055	(.021)**	.082	(.014)**	.056	(.014)	.036	(.012)

Note: significant increases (*) or decreases (**) in jump thresholds are compared with the corresponding score of the 4 mo age group. ($p<.05$, Dunnett comparison).

Table 26: Summary of the Analysis of Variance for Jump Threshold Difference Scores Following Morphine

Condition	SS	df	MS	F-value	p
Age Group (A)	0.36	4	0.09	8.20	<.001
Dose (D)	0.37	3	0.12	49.93	<.001
Time (T)	0.16	3	0.05	22.44	<.001
A x D	0.22	12	0.02	7.55	<.001
A x T	0.08	12	0.01	2.68	<.010
D x T	0.04	9	0.00	4.27	<.001
A x D x T	0.05	36	0.00	1.23	>.050

Figure 18. Alterations (Dunnett comparison) in jump thresholds at 30 min following morphine in different age groups. A difference score analysis was performed because significant differences in vehicle thresholds were observed among age groups.


.20
.16
.12
.08
.04
.0
DIFFERENCE SCORE JUMP THRESHOLD (msec)
1.0
2.5
5.0
10.0
MORPHINE DOSE (mg/kg)
Cohort (mo)
4 mo
9 mo
14 mo
19 mo
24 mo

this was the time period during which the greatest age differences were found. Significant negative correlations between age group and analgesia score were found following the 2.5 ($r=-.399$; $r^2=.159$, $p<.01$), 5.0 ($r=-.470$; $r^2=.221$, $p<.01$), and 10.0 ($r=-.540$, $r^2=.292$, $p<.01$) mg/kg doses. Thus, increase in age is associated with decrease in jump threshold score; this relationship appears to be dose dependent, in that higher correlations were found with higher dosage levels.

Morphine Hyperthermia: Figure 19 and Tables 27 and 28 summarize the alterations in core body temperature following morphine administration. Although all animals showed hyperthermia following all doses, the duration of hyperthermia differed among age groups. The 4, 9 and 14-month age groups displayed similar profiles of hyperthermia with significant increases 60 and 90 min following the 1.0 mg/kg dose, and from 60 to 180 min following the 2.5, 5.0 and 10.0 mg/kg doses, except for a shorter hypothermic effect in the 14-month age group at the lowest morphine dose. The 19-month group displayed increases in core body temperature from 90 to 180 min following the 1.0 mg/kg dose, 60-180 minutes following the 2.5 and 5.0 mg/kg doses, and 90-180 min following the 10.0 mg/kg doses. In contrast, the 24-month age group showed significant morphine hyperthermia 90 min following the 1.0 mg/kg dose, 90 and 180 minutes following the 2.5 dose, and from 60 to 180 min following the 5.0 and 10.0 mg/kg doses. Thus, all groups showed hyperthermia following morphine administration at all doses, though the duration of hypothermia differed as a function of age. With increase in age, there was a tendency for a more persistent

Table 27. Alterations in Core Body Temperature (^{o}C) Following Morphine Across Age Groups.

Age Group / Dose (mg/kg)	Pre	Post-Injection (min) 30	60	90	180
4-month					
0.0	38.17	38.20	38.19	38.16	38.05
	(.122)	(.131)	(.087)	(.098)	(.122)
1.0	38.29	38.54	38.80 *	38.75*	38.23
	(.140)	(.146)	(.217)	(.189)	(.188)
2.5	38.16	38.44	38.99*	38.94*	38.54
	(.139)	(.183)	(.161)	(.138)	(.146)
5.0	38.19	38.54	38.65*	38.92*	38.59*
	(.164)	(.128)	(.133)	(.158)	(.166)
10.0	38.30	38.51	38.92*	39.03*	38.69*
	(.197)	(.222)	(.187)	(.151)	(.139)
9-month					
0.0	38.10	38.17	38.21	38.19	38.17
	(.092)	(.079)	(.064)	(.074)	(.075)
1.0	38.12	38.46	38.58*	38.58*	38.43
	(.088)	(.139)	(.121)	(.128)	(.083)
2.5	38.08	38.44	38.89*	39.03*	38.68*
	(.073)	(.085)	(.094)	(.083)	(.161)
5.0	38.03	38.41	38.83*	38.97*	38.60*
	(.067)	(.098)	(.150)	(.154)	(.210)
10.0	38.27	38.41	38.80*	38.87*	38.64*
	(.110)	(.098)	(.084)	(.162)	(.157)
14-month					
0.0	38.06	38.12	38.09	38.25	38.24
	(.149)	(.103)	(.124)	(.086)	(.110)
1.0	38.04	38.21	38.62*	38.46	38.46
	(.082)	(.143)	(.148)	(.119)	(.103)
2.5	37.99	38.19	38.75*	38.94*	38.90*
	(.099)	(.178)	(.119)	(.156)	(.147)
5.0	37.95	38.49*	38.86*	39.02*	38.82*
	(.127)	(.158)	(.205)	(.150)	(.096)
10.0	37.96	38.39*	38.69*	38.69*	38.65*
	(.144)	(.137)	(.152)	(.168)	(.160)

Table 27 continues on next page

Table 27 (cont.): Alterations in core body temperature (^{o}C) following morphine across age groups.

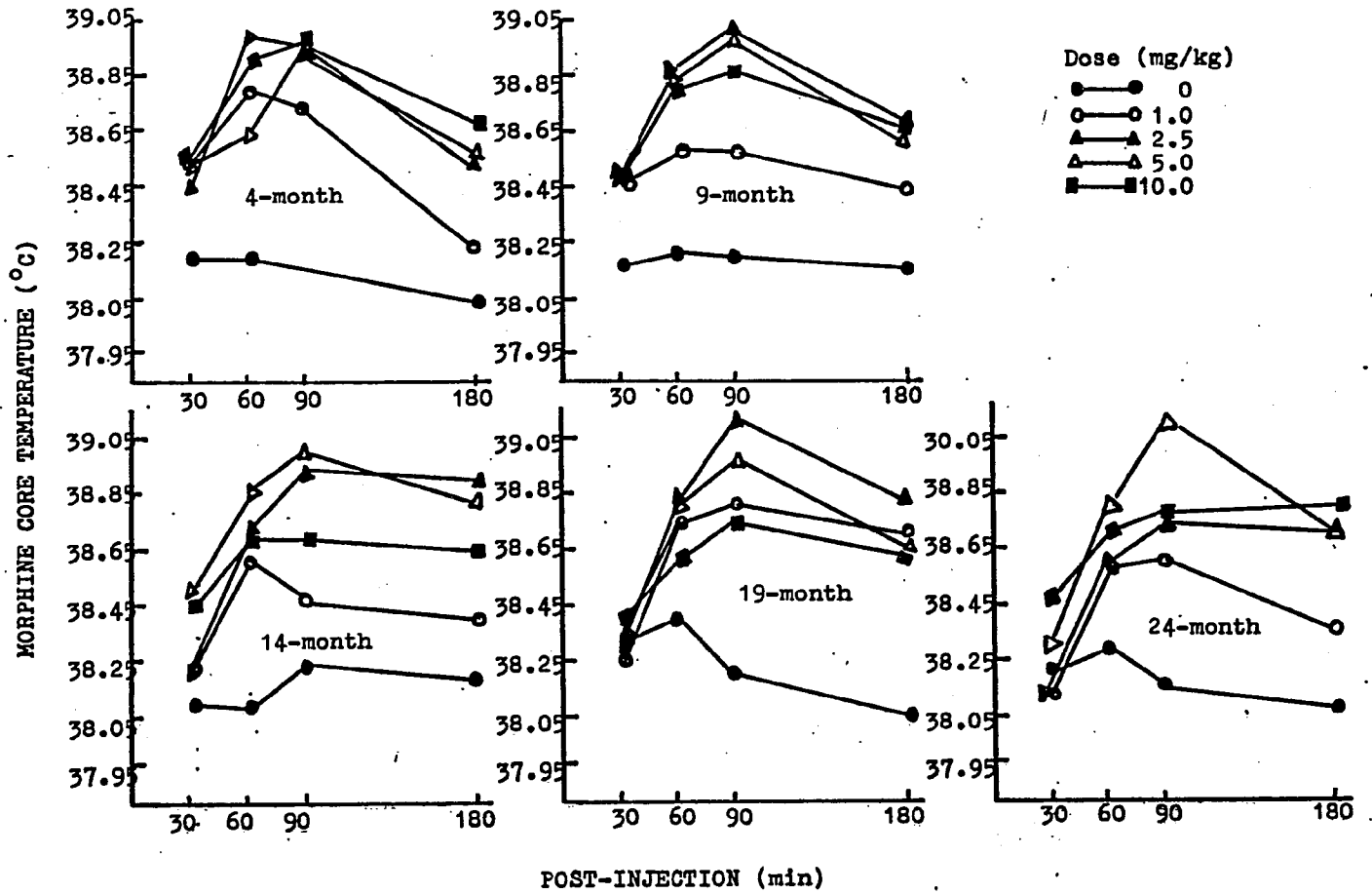
Age Group / Dose (mg/kg)	Pre	Post-Injection (min) 30	60	90	180
19-month					
0.0	38.24	38.31	38.41	38.22	38.05
	(.080)	(.123)	(.139)	(.150)	(.156)
1.0	38.06	38.22	38.74	38.82*	38.72*
	(.105)	(.208)	(.186)	(.168)	(.142)
2.5	38.31	38.26	38.84*	39.14*	38.84*
	(.119)	(.271)	(.147)	(.164)	(.122)
5.0	38.25	38.30	38.80*	38.96*	38.66*
	(.125)	(.128)	(.133)	(.140)	(.157)
10.0	38.07	38.39	38.60	38.76*	38.62*
	(.133)	(.163)	(.175)	(.175)	(.160)
24-month					
0.0	38.22	38.22	38.30	38.17	38.08
	(.251)	(.291)	(.235)	(.250)	(.185)
1.0	38.30	38.13	38.57	38.62*	38.38
	(.181)	(.221)	(.131)	(.108)	(.083)
2.5	38.32	38.12	38.60	38.75*	38.78*
	(.288)	(.197)	(.257)	(.211)	(.170)
5.0	38.23	38.28	38.82*	39.12*	38.78*
	(.229)	(.270)	(.275)	(.430)	(.295)
10.0	38.35	38.47	38.72*	38.78*	38.80*
	(.235)	(.354)	(.228)	(.329)	(.408)

Note: * significant differences ($p<.05$, Dunnett comparison) from corresponding vehicle score.

Table 28: Summary of the Analysis of Variance for Alterations in Core Body Temperature Following Morphine

Condition	SS	df	MS	F-value	p
Age Group (A)	0.79	4	0.12	0.10	>,050
Dose (D)	25.68	4	6.42	19.22	<.001
Time (T)	43.84	4	10.96	79.48	<.001
A x D	2.54	16	0.16	0.48	>.050
A x T	3.90	16	0.24	1.77	<.050
D x T	13.06	16	0.82	9.93	<.001
A x D x T	4.89	64	0.08	0.93	>.050

Figure 19. Alterations in core body temperature during the 180 min interval following morphine in different age groups.

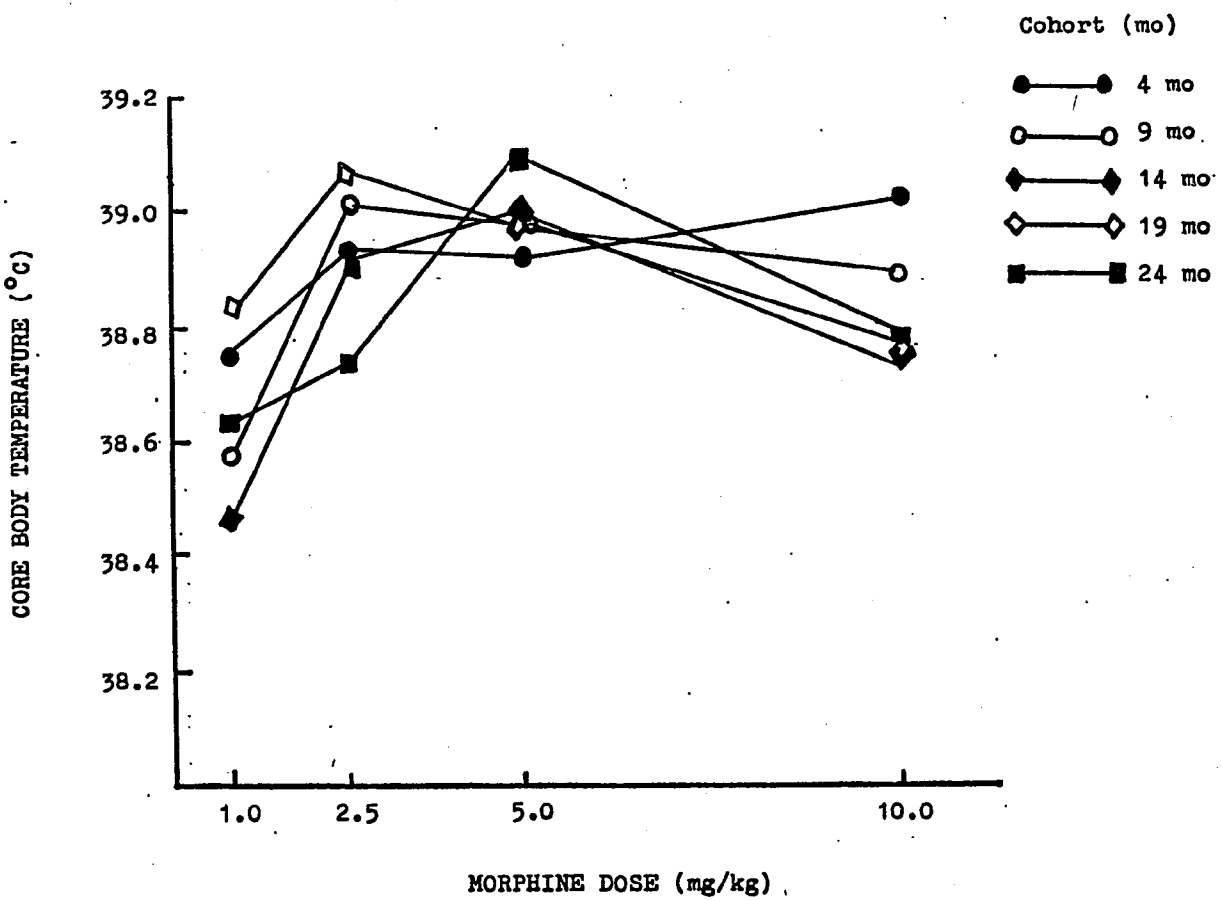

MORPHINE CORE TEMPERATURE (°C)
39.05
38.85
38.65
38.45
38.25
38.05
37.95
30
60
90
180
4-month
9-month
14-month
19-month
24-month
Dose (mg/kg)
0
1.0
2.5
5.0
10.0
POST-INJECTION (min)

hyperthermic response.

With respect to the degree of hyperthermia in younger (4 mo) as opposed to older age groups, Figure 20 shows that similar increases in core body temperature occurred at the 90 min post-injection interval across doses and age groups. This was generally true for the other post-injection intervals as well, except for a small increase in hypothermia in the 14 and 19-month age groups 180 min following the 2.5 and 1.0 mg/kg doses, respectively, and a small reduction in hyperthermia in the 24 month age group 60 min following the 2.5 mg/kg dose. In general, however, the trend was for equivalent levels of hypothermia across age groups. Moreover, the increase in core body temperature across age groups appeared to be independent of the analgesic responses, in that no dose-dependent relationship was found.

To examine the progression of age-related changes in morphine hyperthermia, correlations were computed between the age group and the analgesia difference scores for each morphine dose. None of the correlations were statistically significant, suggesting that the thermic regulatory response to morphine is independent of the age of the animal.

Figure 20. Alterations (Dunnett comparison) in core body temperatures at 90 min following morphine in different age groups.

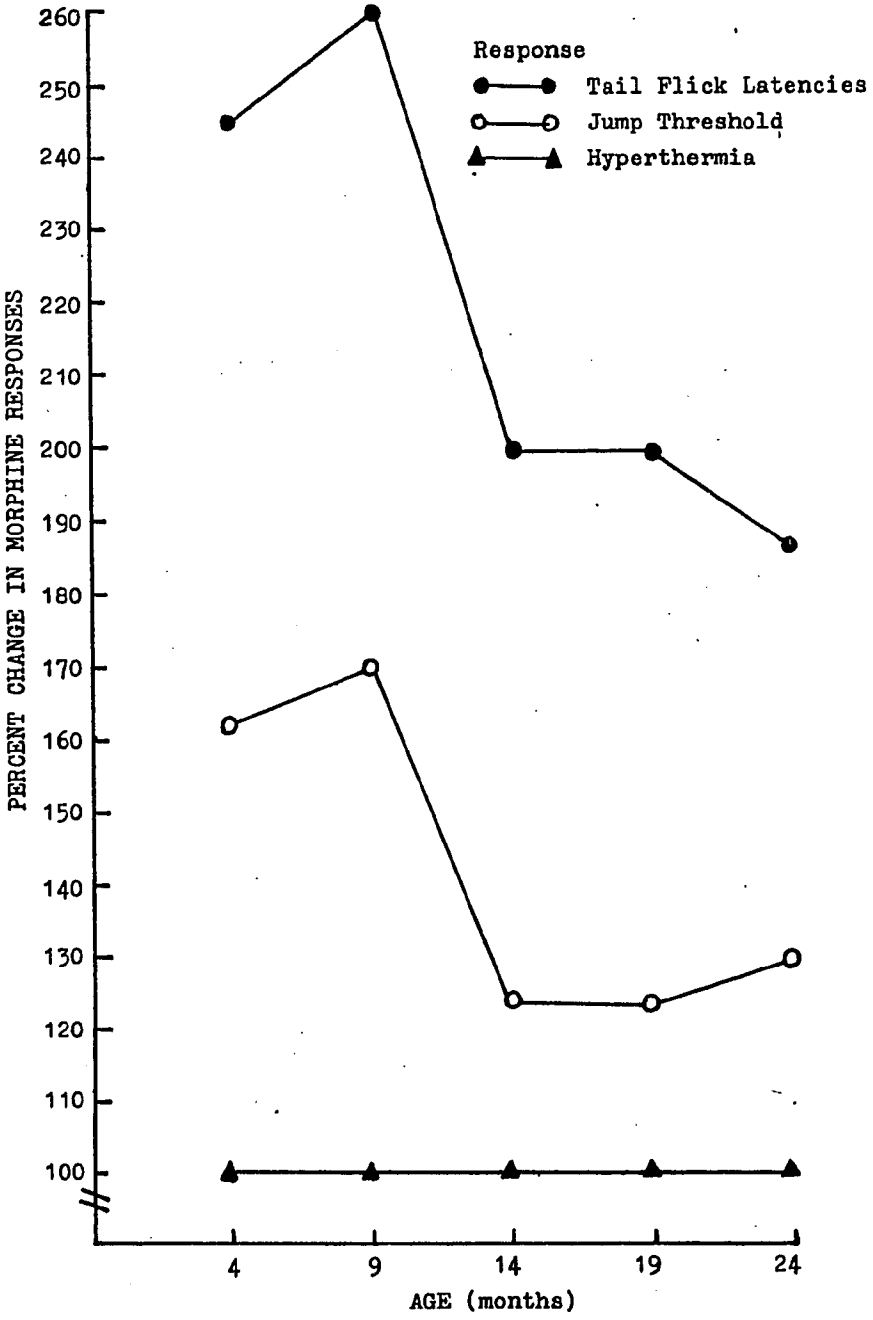

Cohort (mo)
4 mo
9 mo
14 mo
19 mo
24 mo
39.2
39.0
38.8
38.6
38.4
38.2
CORE BODY TEMPERATURE (°C)
1.0
2.5
5.0
10.0
MORPHINE DOSE (mg/kg)

Discussion

Age differences in analgesic responses to morphine were manifested on the tail flick and jump tests. Figure 21 shows that the differential age-related analgesic responses at 30 min following the 10.0 mg/kg doses on the tail-flick and jump tests occurred independently of hypothermic responses, which did not differ as a function of age. The lower analgesic responsivity in the 14-24 month age groups on the tail-flick test was only observed at this early post-injection interval. In contrast, the percentage change in jump thresholds 30 min following the 10 mg/kg dose was 162% in the 4-month group, 169% in the 9-month group, and only 124-129% in the three older age groups. These age-related reduction in jump thresholds in older as compared to younger rats were evidenced across the time course following morphine administration.

The different patterns of morphine analgesia exhibited on the tail-flick and jump tests may be due to a variety of factors. For instance, the failure to find age-related changes in morphine analgesia on the tail-flick test may be related to the 6-sec cutoff criterion. Specifically, since the majority of animals reached the ceiling criterion, the extent of analgesia above this point could not be assessed. Alternatively, the differences between tests may reflect spinal mediation of the tail flick test as compared to supraspinal mediation on the jump test. In this regard, morphine analgesia as measured by mechanical and thermal pain tests, is modulated by different central processes. Intrathecal administration of the serotonin neurotoxin,

Figure 21. Percent change in tail-flick latency, jump threshold and hyperthermic responses 30 min following a 10.0 mg/kg morphine dose in different age groups.


Response
Tail Flick Latencies
Jump Threshold
Hyperthermia
PERCENT CHANGE IN MORPHINE RESPONSES
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
4
9
14
19
24
AGE (months)

5,6-dihydrotryptamine, but not the catecholamine, neurotoxin 6-hydroxydopamine, has been shown to inhibit morphine analgesia on the hot plate test (Kuraishi, Harada, Aratini, Satoh, and Takagi, 1983). In contrast, only 6-hydroxydopamine reduced morphine analgesia on the tail pinch test. Moreover, as neither of these manipulations affected morphine analgesia on the tail flick test, the latter effect was attributed to its direct spinal action (Yaksh, 1981).

The results of the present experiment agree with some, but not all of the reported results of studies of age-related changes in morphine analgesia. Again, some of the discrepant findings may be attributable to a lack of consistency in the definition of "old" groups, to the fact that different species were used in different studies, and/or to the use of different pain-inducing stimuli and different response measures. For example, Chan and Lai (1982) reported that older rats showed reductions in morphine (10mg/kg) and clonidine analgesia on the hot plate test, which were attributed to age-related reductions in the concentrations of dopamine, acetylcholine and opiate receptors. However, it is difficult to compare the results of that study with the present results since their groups were 2, 5, and 11 months of age, respectively. The present study found no difference between 4- and 9-month old groups, although a reduction in jump thresholds was observed by 14 months of age. Another difficulty in comparing the Chan and Lai (1982) study with the present one is that morphine analgesia on the hot plate test is modulated by different processes from that of the tail flick or jump test (Kuraishi et al, 1983).

Although age differences in dose-related alterations in morphine analgesia on the tail flick test have been reported (Spratto and Dorio, 1978), differences between that study and this one in age groups make comparisons difficult. Spratto and Dorio (1978) reported that younger rats (1.5 mo) displayed more analgesia following a 5 mg dose of morphine than older rats (6,10 mo), but the converse was true for a 7.5 mg/ kg dose. Moreover, age-related comparisons for the low (3.0) and high (10.0) doses were affected by variance and ceiling criteria, respectively. However, the results of the present study agree with those reporting a reduction in morphine analgesia for only 30 min after morphine (5 mg/kg) administration on both the tail flick and hot plate tests in mice ranging from 2 to 28 months of age. As with our study, no consistent age differences in morphine analgesia were found for post-injection intervals longer than 30 min (Webster, Shuster and Eleftheriou, 1976; Kavaliers, et al., 1983). The only previous study in which morphine analgesia on the jump test as a function of age was investigated (Saunders, Paolino, Bousquet, Miya, 1974); reported that morphine analgesia was greater in older (9-10 months) than in younger (2.5-3 months) rats. Again, the different response measure (vocalization) and the gender used (males) differed from those in the present study. The present results agree with reported findings of age-related reductions in another form of opioid-mediated analgesia-front paw footshock analgesia- which is progressively reduced with increase in age (5-24 mo) (Hamm and Knisley, 1985) and is attenuated following nalaxone pretreatment (Watkins and Mayer, 1982). In summary, most studies have examined

morphine analgesia in response to a thermal pain stimulus (tail flick or hot plate), and have generally reported reduced analgesia in older rodents. However, the reduction in morphine analgesia across studies are only apparent within a 30 min period after drug administration. Consequently, the results of these studies agree with those of the present one in that morphine analgesia was reduced on the tail-flick test in the older age groups (14, 19 and 24-months) relative to the younger groups (4 and 9 months) at only the 30 min post-injection interval. However, thereafter significant analgesia was observed for rats in all age groups. Prolonged age-related reductions in morphine analgesia were only apparent on the jump test, and so suggest the need for evaluation of morphine analgesia on a variety of pain tests concurrently in the same animals. Indeed, these studies point to the diversity of actions of morphine, i.e., that such factors as the time period following drug administration, the doses used, and the pain measures used, contribute to different patterns of analgesic responsivity as a function of age. Similarly, inconsistent reports exist regarding morphine analgesia in elderly humans. Although some report on increased morphine pain relief for acute postoperative pain (Bellville et al., 1971), others suggest that this merely refelcts a greater tendency on the part of older people to responsd to placebos than younger people (Lasagna, 1971).

The observed age-related reductions in morphine-induced analgesia on the jump test parallel the reductions in opiate binding sites and opioid levels in the aging rodent. There are reduced beta-endorphin concentrations in the hypothalamus and

striatum, and reduced opiate receptor concentrations in the frontal cortex, striatum, amygdala, and hippocampus (Barden et al, 1981; Gambert et al, 1980; Hess et al, 1980). In addition, there are reductions in the concentrations of met and leu-enkephalin in the suprachiasmatic, arcuate, premamillary, medial preoptic, paraventricular and ventromedial nuclei, and reduced opiate receptor binding and affinity in the thalamus, midbrain and cortex of aging rodents (Dupont et al., 1981, Messing et al, 1980; Jensen et al, 1980). Other factors contributing to reduced morphine analgesia with increase in age are those associated with the reduced opioid levels in the aging rodent brain, including decreases in immunoreactive ACTH levels, and less efficient interactions with the thyroid, tubero-infindibular dopaminergic and gonadal reproductive systems (Barden et al., 1981; Gambert et al., 1980, 1981; Kumar et al., 1980).

The cholinergic system may also be involved in the decrease in morphine analgesia in aging rats. Acetylcholine and morphine potentiate each other's morphine analgesic effects as well as develop partial cross-tolerance (Pedigo and Dewey, 1981;, Takemori et al., 1975). Moreover, the anatomical distribution of central injection sites for acetylcholine- induced analgesia is similar to that of morphine and stimulation-produced analgesia (Pert and Maxey, 1975; Pedigo and Dewey, 1981). In the aging rodent, reduced acetylcholine levels, as well as its synthesizing enzymes, precursors, and receptor binding sites have been reported for the whole brain, striatum, hippocampus, cortex and cerebellum (Meek et al., 1977; McGeer, 1971; Morin and Wasterlain, 1980; Jensen, 1978;

Ordy and Schjeide, Freund, 1980, Moudgil and Kanungo, 1973).

In the current experiment, morphine hyperthermia was present in all age groups for all morphine doses. In general, there was no no significant age-or-dosage differences, although there was a general trend for longer duration of the hyperthermia at the lower doses by the older age groups. The time course for morphine hyperthermia (maximum 60-180 min) and lack of dose-response effects are consistent with the findings of other investigators (Lotti et al., 1965; Herman, 1942), which suggest that morphine hyperthermia is relatively independent of morphine analgesia. Thus, the reduced analgesic response seen in older animals probably reflects morphine's specific modulation of pain inhibitory processes.

GENERAL DISCUSSION

The results of the three experiments indicate that: 1. the analgesic responses following morphine, CWS and 2DG are all reduced with increases in age, but the pattern of decline differs for each manipulation; 2. the reduced adaptive responsivity of older animals does not appear to reflect a generalized deficit, in that there are different patterns of responsivity on other concomittant physiological measures; and 3. although a causal relationship cannot be established from the data of the present experiments, it would appear that the age-related declines in adaptive responses parallel the reduced functioning of the hypothalamo-pituitary-adrenal, catecholamine, opioid and cholinergic systems.

When behavioral changes are found as a function of age, at least three developmental curves can describe the effects: 1) a progressive developmental trend; 2) a gradual increase or decrease in certain functions that return to an earlier form at later stages or 3) a sudden decline in an otherwise stable function (Jensen et al., 1980). The last possibility may reflect either the natural aging process (eugeric changes), or the influence of secondary factors such as disease or pathology (pathogeric) (Lytle and Altar, 1979; Finch et al., 1973). The age-related adaptive behaviors examined in the present study appear to reflect the mediation of all three developmental curves. The analgesic adaptive response to 2DG, for example, reflects the first type of change. Both tail flick and jump threshold measures showed a progressive reduction as a function of age, beginning with a reduction in responsivity at 14 months and

culminating in a total elimination of the response by 24 months of age. Moreover, the mechanisms mediating this decline may also parallel the stress-induced hyperphagic response, as a similar progressive decline in food intake was found with increase in age. In contrast, age differences in morphine analgesia appear to reflect the second type of developmental trend. With increasing age morphine analgesia showed a sudden decline, but a trend towards recuperation of the response was evidenced in the oldest age group. Finally, CWS analgesia appears to reflect the third mechanism of action; sudden decline at 24-months of age in analgesic responsiveness on the tail flick test was found in an otherwise developmentally stable function.

The question of whether the age-related changes are a function of natural aging (eugeric) or due to secondary factors as disease processes (pathogeric) remains. The present experiment did not make use of pathological indices, but, nevertheless, on a behavioral level, evidence seems to argue against an exclusive pathogeric function. For example, in the 2DG experiment, the reduction in analgesia and hyperphagia was not an abrupt phenomena, but instead was characterized by a gradual reduction beginning at 14 mo of age . Although an abrupt reduction in analgesia was seen on the jump test following morphine administration (14 mo), the data argue against a pathogeric influence, since analgesia was observed on the tail-flick test. The gradual recovery of responsivity with increase in age adds further support for eugeric rather than pathogeric changes. In addition, thermal responsivity to morphine was not differentially affected by age. The strongest argument for a pathogeric change would seem to arise from the results of the CWS experiment. In this case, a rather sudden

decline (24 mo) in analgesia was observed on the tail-flick test. However, this occurred only following the 2°C swim condition, whereas no differences were observed at other water temperatures. Moreover, this was accompanied by an increase rather than a decrease in hypothermic responsivity.

Regardless of whether eugeric (age-related) rather than pathogeric (disease) mechanisms are involved, the issue of whether aging itself is associated with generalized rather than specific deficits can be raised. The present results argue for differential age-related changes in that hyperresponsivity rather than hyporesponsivity to painful stimulation was the general trend with increasing age. Moreover, a multiplicity of mechanisms underlying coping responses is suggested by the fact that CWS analgesia, morphine analgesia and 2DG analgesia are differentially attenuated with age, and in themselves are modulated by different mechanisms.

Since age-related, rather than pathogeric, mechanisms that reflect specific, rather than generalized, deficits, may be involved, the question arises as to which systems are mediating the three different patterns of analgesic declines. Although 2DG, CWS, and morphine analgesia share common properties, morphine has usually been classified as being primarily opioid mediated, CWS as nonopioid mediated, and 2DG as having both opioid and nonopioid properties (see review: Watkins and Mayer, 1982; Bodnar et al., 1980). In this regard, it is of interest that the onset of analgesic decline as a function of age was more similar for morphine and 2DG (14 mo) than for CWS (24 mo). However, as a variety of other manipulations affect 2DG and CWS analgesia in both similar and different ways as that of

morphine analgesia, it appears that age related changes in analgesic respnosivity do not merely reflect the action of a specific pain inhibitory system. The mechanisms by which age changes in animals occur cannot be discerned from the present data, only that covariance among the different systems affected by aging may be involved. For example, the hypothalamic-pituitary-adrenal axis appears to modulate certain analgesic responses. Hypophysectomy attenuates CWS analgesia, but potentiates morphine and 2DG analgesia, and the adrenal cortex modulates both morphine and CWS analgesia (Holoday et al., 1978; 1979, Bodnar et al, 1979). In this regard, neuroendocrine system changes have been postulated to contribute to the loss of ability to adjust to internal and external environmental changes with increasing age. For example, according to the "hypothalamic dysregulation" theory (Frolkis and Betzukov, 1979), the aging of the hypothalamus involves a differential decline in the functioning of individual hypothalamic nuclei. Consequently, one would expect a variety of changes in different physiological systems that are regulated by the hypothalamus. Other theorists have formulated a more unitary view, and point to a general reduction in the sensitivity of the hypothalamus to negative feedback that serves to disrupt communication between the hypothalamus and other neural and endocrine systems (Dilman, 1976). The differential age-related changes found in the present study are more supportive of the former rather than of the latter position.

Still other theorists point to a neurotransmitter deficit as modulating the reduced ability to adapt to stress with increasing age. Relative inefficient functioning has been reported for a number of

neurotransmitter systems, particularly the catecholamine and cholinergic systems (Finch et al, 1973; Freund, 1980). Further evidence has come from the observations that treatment with dopamine precursors, monoamine oxidase inhibitors, or serotonin antagonists, have been found to delay the onset of aging (Clemens and Fuller, 1977; Cotzias et al, 1977; Segall, 1979). With respect to stress-induced analgesia, however, the catecholaminergic system appears to play a diverse role. Whereas dopamine blockade or decreased availability increases CWS and 2DG analgesia, only morphine analgesia is reduced following depeletion of serotonin (Tenen, 1968; Samanin et al, 1973; Bodnar et al, 1981). On the other hand, clonidine and desipramine pretreatment increase CWS analgesia (Bodnar et al, 1983; 1985). According to an alternative neurotransmitter model, it is the imbalance among neurotransmitter systems in particular brain areas that are associated with age-related behavior changes, and this imbalance is accentuated in response to stress (Algeri et al., 1982; Timiras, 1982).

In conclusion, the diversity of changes in age-related adaptive behaviors found in the present study is in agreement with the empirical findings reported in the literature regarding reductions in hypothalamic-pituitary-adrenal interactions, catecholamine neurotransmitter levels, and opioid levels. However, for the most part, direct comparisons between the results of the present experiments and those of other investigators are made difficult as the neurochemical indices across studies differ with regard to age, species, or gender used. Although the mechanisms by which age changes in analgesia occur cannot be discerned from the present data, the lack

of covariance between different stress responses found in the present study on a behavioral level suggest that aging in the rodent is associated with specific rather than generalized deficits that probably reflect an imbalance among systems and different processes of change with change in age.

References

Akil, H., Madden, J., Patrick, L., & Barchas, P.D. (1976). Stress-induced increases in endogenous opiate peptides: concurrent analgesia and its partial reversal by nalaxone. In H.W. Kosterlitz (Ed), Opiates and Endogenous Opiate Peptides. Amsterdam: North Holland.

Akil, H., & Mayer, D.J. (1972). Antagonism of substance P by P-CPA, a serotonin synthesis inhibitor. Brain Research, 44, 692-696.

Akil, H., Mayer, D.J., & Liebeskind, J.C. (1976). Antagonism of stimulation produced analgesia by nalaxone, a narcotic antagonist. Science, 191, 961-962.

Algeri, S., Calderini, G., Lomuscio, G., Vantini, G., Toffano, G., Ponzio,F. Changes with age in rat central monoaminergic system responses to cold stress. Neurobiology of Aging, 1982, 3, 237-242.

Amir, S., & Amit, Z. (1978). Endogenous opioid ligands may mediate stress-induced changes in the affective properties of pain related behavior in rats. Life Sciences, 23, 1143-1152.

Axelrod, J., & Reisine, T.D. (1984). Stress hormones: their interaction and regulation. Science, 224, 452-459.

Badillo-Martinez, D., Nicotera, P.D., Butler, P.D., Kirchgessner, A.L., & Bodnar, R.J. (1984). Impairments in analgesic, hypothermic and glucoprivic stress responses following neonatal

monosodium glutamate. Neuroendocrinology, 33, 438-446.

Badillo-Martinez, D., Nicotera, P.D., Butler, P.D., Kirchgessner, A.L., Sperber, E., & Bodnar, R.J. (1982). Characterization of stress-related responses in the monosodium glutamate treated rat. Society Neuroscience Abstract, 8, 621.

Balgura, S., & Ralph, T. (1973). The analgesic effect of electrical stimulation of the diencephalon and mesencephalon. Brain Research, 60, 369-379.

Barden, N., Dupont, A., Labrie, F., Merand, Y., Rouleau, D., & Vaudry, H. (1981). Age-dependent changes in the B-endorphin content of discrete rat brain nuclei. Brain Research, 208, 209-212.

Basbaum, A.I., & Fields, H.L. (1984). Endogenous pain control systems: Brainstem spinal pathways and endorphin circuitry. Annual Review Neuroscience, 7, 309-338.

Basbaum, A.I., Marley, N.J.E., O'Keefe, J., & Clanton, C.H. (1977). Reversal of morphine and stimulation-produced analgesia by subtotal spinal cord lesions. Pain, 3, 43-56.

Belluzi, J.D., Grant, N., Garsky, V., Sarantakis, D., Wise, C.D., & Stein, L. (1976). Analgesia induced in vivo by central administration of enkephalin in rat. Nature, 260, 625-626.

Bellville, J.W. (1971). Influence of age on pain relief from analgesics. J. Amer. Med. Assoc., 217, 1835-1841.

Berthoud, H.R., & Mogenson, G.J. (1977). Ingestive behavior after intracerebral and intracerebroventricular infusions of glucose

and 2-deoxy-D-glucose. American Journal of Physiology, 233, 127-133.

Bodnar, R.J. (1984). Types of stress which induce analgesia. In M.D. Tricklebank and G. Curzon (Eds.), Stress-Induced Analgesia, NY: John Wiley & Sons.

Bodnar, R.J., Abrams, G.W., Zimmerman, A.E., Krieger, D.T., Nicholson, G., & Kizer, J.S. (1980). Neonatal monosodium glutamate: effects upon analgesic responsivity and immunocytochemical ACTH/B-Lipotropin. Neuroendocrinology, 30, 280-284.

Bodnar, R.J., Glusman, M., Brutus, M., Spiaggia, A., & Kelly, D.D. (1979). Analgesia induced by cold-water stress: attenuation following hypophysectomy. Physiology and Behavior, 23, 53-62.

Bodnar, R.J., Kelly, D.D., & Glusman, M. (1978). Stress-induced analgesia: time course of pain reflex alterations following cold-water swims. Bulletin of the Psychonomic Society, 11, 333-336.

Bodnar, R.J., Kelly, D.D., & Glusman, M. (1979) 2-deoxy-D-glucose analgesia: influences of opiate and non-opiate factors. Pharmacology, Biochemistry and Behavior, 11, 297-301.

Bodnar, R.J., Kelly, D.D., Brutus, M., & Glusman, M. (1978). Chronic 2-deoxy-D-glucose treatment: adaptation of its analgesic, but not hyperphagic properties. Pharmacology, Biochemistry and Behavior, 9, 763-768.

Bodnar, R.J., Kelly, D.D., Brutus, M., & Glusman, M.

Stress-induced analgesia: neural and hormonal determinants. (1980). Neuroscience and Biobehavioral Review, 4, 87-100.

Bodnar, R.J., Kelly, D.D., Brutus, M., Greenman, C.B., Glusman, M. Reversal of stress-induced analgesia by apomorphine, but not by amphetamine. Pharmacology, Biochemistry and Behavior, 1980, 13, 171-175.

Bodnar, R.J., Kelly, D.D., Brutus, M., Mansour, S., & Glusman, M. (1978). 2-Deoxy-D- glucose-induced decrements in operant and reflex pain thresholds. Pharmacology, Biochemistry and Behavior, 9, 543-549.

Bodnar, R.J., Kelly, D.D., & Glusman, M. (1979). 2-Deoxy-D-glucose analgesia: influences of opiate and non-opiate factors. Pharmacology, Biochemistry and Behav., 11, 297-301.

Bodnar, R.J., Kelly, D.D., Mansour, S., & Glusman, M. (1979). Differential effects of hypophysectomy upon analgesia induced by two glucoprivic stressors and morphine. Pharmacology, Biochemistry and Behavior, 11, 303-307.

Bodnar, R.J., Kelly, D.D., Spiaggia, A., Ehrenberg, C., & Glusman, M. (1978). Dose- dependent reductions by nalaxone of analgesia induced by cold-water stress. Pharmacology, Biochemistry and Behavior, 8, 667-672.

Bodnar, R.J., Kelly, D.D., Spiaggia, A., & Glusman, M. (1978). Stress-induced analgesia: adaptation following cold-water swims. Bulletin of the Psychonomic Society, 11, 337-340.

Bodnar, R.J., Kelly, D.D., Steiner, S.S., & Glusman, M. (1978).

Stress-produced analgesia and morphine-produced analgesia: lack of cross tolerance. Pharmacology, Biochemistry and Behavior, 8, 661-666.

Bodnar, R.J., Kordower, J.H., Wallace, M.M., & Tamir, H. (1981). Stress and morphine analgesia: alterations following p-chlorophenylalanine. Pharmacology, Biochemistry and Behavior, 14, 645-651.

Bodnar, R.J., Kramer, E., Simone, D.A., Kirchgessner, A.L., & Scalisi, J. (1983). Dissociation of analgesic and hyperphagic responses following 2-deoxy- D-glucose. International Journal Neuroscience, 21, 225-236.

Bodnar, R.J., Lattner, M., & Wallace, M.M. (1980). Antagonism of stress-induced analgesia by D-phenylalanine, an anti-enkephalinase. Pharmacology, Biochemistry and Behavior, 13, 829-833.

Bodnar, R.J., Mansour, A., & Glusman, M. (1979). Differential effects of hypophysectomy upon analgesia induced by two glucoprivic stressors and morphine. Pharmacology, Biochemistry and Behavior, 11, 303-308.

Bodnar, R.J., Mann, P.E., & Stone, E.A. (1985). Potentiation of cold-water swim analgesia by acute, but not chronic desipramine administration. Pharmacology, Biochemistry and Behavior, in press.

Bodnar, R.J., Merrigan, K.P., & Sperber, E.S. (1983). Potentiation of cold-water swim analgesia and hypothermia by clonidine.

Pharmacology Biochemistry and Behavior, 19, 447-451.

Bodnar, R.J., Merrigan, K.P., & Wallace, M.M. (1981). Analgesia following intraventricular administration of 2-deoxy-D-glucose. Pharmacology, Biochemistry and Behavior, 14, 579-581.

Bodnar, R.J., & Nicotera, N. (1982). Neuroleptic and analgesic interactions upon pain and activity measures. Pharmacology, Biochemistry and Behavior, 16, 4-11.

Bodnar, R.J., Sikorszky, V. (1983). Nalaxone and cold-swim analgesia: parametric considerations and individual differences. Learning and Motivation, 14, 223-237.

Bodnar, R.J., Wallace, M.M., Kordower, J.H., Nilaver, G., Cort, J., & Zimmerman, E.A. (1982). Modulation of nociceptive thresholds in Brattleboro and normal rats. Annals of the NY Academy of Science, 394, 735-739.

Bodnar, R.J., Zimmerman, E.A., Nilaver, G., Mansour, A., Thomas, L.W., Kelly, D.D., & Glusman, M. (1980). Dissociation of cold water swim and morphine analgesia in Brattleboro and normal rats with diabetes insipidus. Life Sciences, 26, 1581-1590.

Bolles, R.L., & Fanselow, M.S. (1982). Endorphins and behavior. Annual Review Psychology, 35, 87-101.

Bonnet, K.A., & Peterson, K.E. (1975). A modification of the jump-flinch technique for measuring pain sensitivity in rats. Pharmacology, Biochemistry and Behavior, 3, 47-55.

Bracho-Romero, E., & Reaven, G.M. (1977). Effect of age and weight on plasma glucose and insulin responses in the rat. Journal of

the American Geriatrics Society, 25, 299-302.

Brady, K., Brown, J.W., & Thurmond, J.B. (1980). Behavioral and neurochemical effects of dietary tyrosine in young and aged mice following cold-swim stress. Pharmacology, Biochemistry and Behavior, 12, 667-674.

Brett, L.P., Chong, G.S., Coyle, S., & Levine, S. (1983). The pituitary-adrenal response to novel stimulation and ether stress in young adult and aged rats. Neurobiology of Aging, 4, 133-138.

Britton, G.W., Rotenberg, S., & Adelman, R. (1975). Impaired regulation of corticosterone levels during fasting in aging rats. Biochemical, Biophysical Research Communications, 64, 184-188.

Brown, J. (1962). Effects of 2-deoxyglucose on carbohydrate metabolism: a review of literature and studies in the rat. Metabolism, 11, 1098-1112.

Bruni, J.F., Huang, H.H., Marshall, S., & Meites, J. (1977). Effects of single and multiple injections of synthetic GnRH on serum LH, FSH, and testerone in young and old male rats. Biol. Reprod., 17, 309-321.

Brutus, M., Kelly, D.D., Glusman, M., & Bodnar, R.J. (1979). Periaqueductal gray lesions and non-narcotic anti-nociception. Sosciety of Neuroscience Abstracts, 5, 606.

Buck, S.H., Deshmukh, P.P., Burks, T.F., & Yamamura, H.I. (1981). A survey of substance p, somatostatin, and neurotensin levels in

aging in the rat and human central nervous system. Neurobiology of Aging, 2, 257-264.

Burchinsky, S.G. (1984). Neurotransmitter receptors in the central nervous system and aging: pharmacological aspects. Experimental Gerontology, 19, 227-239.

Cannon, J.T., Prieto, G.J., Lee, A., & Liebeskind, J.C. (1982). Evidence for opioid and nonopioid forms of stimulation-produced analgesia in the rat. Brain Research, 243, 315-321.

Chan, S.H.H., & Lai, Y.Y., (1982). Effects of aging on pain responses and analgesic efficacy of morphine and clonidine in rats. Experimental Neurology, 75, 112-119.

Chiueh, C.C., Nespor, S.M., & Rapoport, S.I. (1980). Cardiovascular, sympathetic and adrenal cortical responsiveness of aged fischer-344 rats to stress. Neurobiology of Aging, 1, 157-163.

Chouknyiska, R., & Bassileva-Popova, J.G. (1977). Effect of age on the bonding of H3 testerone with receptor protein from rat brain and testes. C.R. Acad. Bulg. Sci. , 30, 133-135.

Clark, W.C., & Mehl, L. (1971). A sensory decision theory analysis of the effect of age and sex on d', various response criteria, and 50% pain threshold. Journal of Abnormal Psychology, 78, 202-212.

Cooper, R.L., McNamara, M.C., & Thompson, W.G. (1980). Vasopressin and conditioned flavor aversion in aged rats. Neurobiol of Aging, 1, 53-57.

D'Amour, F.E., & Smith, D.L. (1941). A method for determining loss of pain sensation. Journal of Pharmacology and Experimental Therapeutics, 72, 74-79.

DeBlaski, A., Cotecchia, S., & Menini, T. (1982). Selective changes of receptor binding in brain regions of aged rats. Life Science, 31; 335-340.

DeFronzo, R.A. (1981). Glucose intolerance and aging. Diabetes Care, 4, 493-501.

Denckla, W.D. (1974). Role of the pituitary and thyroid glands in the decline of minimal O2 consumption with age. Journal of Clinical Investigations, 53, 572-581.

Dilman, V.M. (1971). Age-associated elevation of hypothalmic threhsold to feedback control, and its role in development, aging and disease. Lancet, I, 1211-1219.

Dilman, V.M. (1976). The hypothalamic control of aging and age-associated pathology: The elevation mechanism of aging. In A.V. Everitt and J.A. Burgess (Eds.) The Hypothalmus, Pituitary and Aging, Springfield, IL.: C.C. Thomas, pp 634-667.

Dilman, V.M., & Anisimov, V.N. (1979). Hypothalamic mechanisms of ageing and of specific age pathology - I. sensitivity threshold of hypothalamo-pituitary complex to homeostatic stimuli in the reproductive system. Experimental Gerontology, 14, 161-74.

Dilman, V.M., Bobrov, J.F., Ostroumova, M.N., Evovich, E.G., Vishnevsky, A.S., Anisimov, V.N., & Vasiljev, I.A. (1979). Hypothalamic mechanisms of ageing and of specific age pathology

- III. sensitivity threshold of hypothalamo- pituitary complex to homeostatic stimuli in energy systems. Experimental Gerontology, 14, 217-24.

Dilman, V.M., Ostroumova, M.N., & Tsyrlina, E.V. (1979). Hypothalmic mechanisms of ageing and of specific age pathology-II. on the sensitivity threshold of hypothalmo-pituitary complex to homeostatic stimuli in adaptive homeostasis. Experimental Gerontology, 14, 175-81.

Dostrovsky, J.O., & Deakin, J.F.W. (1977). Periaqueductal gray lesions reduce morphine analgesia in the rat. Neuroscience Letters, 4, 99-103.

Dupont, A., Savard, P., Merand, Y., Labrie, F., & Boissier, J.R. (1981). Age-Related changes in central nervous system enkephalins and substance P. Life Science, 29, 2317-322.

Dupont, A., Cusan, L., Labrie, F., Coy, D.H., & Li, C.H. (1977). Stimulation of prolactin release in the rats by intraventricular injections of beta- endorphin methionine enkephalin. Biochemical and Biophysical Research Communications, 75, 76-82.

Engeset, R.M., & Ritter, R.C. (1980). Intracerebroventricular 2-DG causes feeding in the absence of other signs of glucoprivation. Brain Research, 202, 229-233.

Epstein, M.H., & Barrows, C.H.J. (1969). The effects of age on the activity of glutamic acid decarboxylase in various regions of the brains of rats. Journal of Gerontology, 24, 136-139.

Estes, K.S., & Simpkins, J.W. (1980). Age-related alterations in

catecholamine concentrations in discrete preoptic areas and hypothalamic regions in the male rat. Brain Research, 194, 556-60.

Evans, W.O. (1961). A new technique for the investigation of some analgesic drugs on reflexive behaviors in the rat. Psychopharmacology, 2, 318-325.

Fields, H. L., & Anderson, B.D. (1978). Evidence that raphe spinal neurons mediate opioid and midbrain stimulation induced analgesia. Pain, 5, 333-349

Fields, H. L., & Basbaum, A. I. (1978). Brainstem control of spinal pain transmission neurons. Annual Review of Physiology, 40, 217-248.

Finch, C.E. (1973). Catecholamine metabolism in the brain of aging male mice. Brain Research, 52, 261-76

Finch, C.E. (1972). Enzyme activities, gene function and ageing in mammals. Experimental Gerontology, 7, 53-59.

Finch, C.E., Foster, J.R., & Mirsky, A.E. (1969). Aging and the regulation of cell activities during exposure to cold. J Gen. Physiol., 54, 690-712.

Fliers, E., & Swaab, D.F. (1983). Activation of vasopressinergic and oxytocinergic neurons during aging in the wistar rat. Peptides, 4, 165-170.

Fonda, M.L., Acree, D.W., & Averbach, S.B. (1973). The relationship of Y-amminobutyrate levels and its metabolism to age in brains of mice. Archives of Biochem. and Biophysics, 159, 622-628.

Forman, L.J., Sonntag, W.E., Van Vugt, D.A., & Meites, J. (1981). Immunoreactive B-endorphin in the plasma, pitutitary and hypothalmis of young and old male rats. Neurobiology of Aging, 2, 281-284.

Freund, G. (1980). Cholinergic receptor loss in brains of aging mice. Life Sciences, 26, 371-375.

Frederickson, R.A., Burgis, V., Harrell, C.E., & Edwards. J.O. (1978). Dual actions of Substance P on nociception: possible role of opioids. Science, 199, 1359-1362.

Frolkis, V.V., Bezrukov, V.V., Duplenko, Y.K., & Genis, E.D. (1972). The hypothalmus in Aging. Experimental Gerontology, 7, 169-184.

Gambert, S.R. (1981). Interaction of age and thyroid hormone status on beta-endorphin content in rat corpous striatum and hypothalamus. Neuroendrocrinology, 32, 114-117.

Gambert, S.R., Garthwaite, T.L., Pontzer, C.H., & Hagen, T.C. (1980). Age-Related changes in central nervous sytem beta-endorphin and ACTH. Neuroendocrinology, 31, 252-255.

Giradot, M-N., & Holloway, F.A. (1984). Intermittent cold water stress-anlgesia in rats: Cross tolerance to morphine. Pharmacology, Biochemistry and Behavior, 20, 631-633.

Giradot, M-N., & Holloway, F.A. (1985). Effect of age and long-term stress experience on adaptation to stress-analgesia in mature rats: role of opioids. Behav. Neurosci., in press.

Giradot, M-N., & Holloway, F.A. (1985). Naltrexone antagonizes the biobehavioral adaptation to cold-water stress in rats.

Pharmacology, Biochemistry and Behavior, in press.

Giradot, M-N., & Holloway, F.A. (1985). Chronic stress, aging and morphine analgesia: chronic stress affects the reactivity to morphine in young mature but not old rats. J. Pharmac. Exp. Ther., in press.

Glusman, M., Bodnar, R.J., Mansour, A., & Kelly, D.D. (1980). Enhancement of stress-induced analgesia by adrenelectomy in the rat. Society for Neuroscience Abstracts, 6, 321.

Gold, P.E. (1984). Age-related changes in brain catecholamine responses to a single footshock. Neurobiology of Aging, 5, 55-59.

Goldstein, A. (1976). Opioid peptides (endorphins) in pituitary and brain. Science, 173, 1081-1086.

Gordon, W.C., Scobie, S.R., & Frankl, S.E. (1978). Age-related differences in electric shock detection and escape thresholds in Sprague-dawley albino rats. Experimental Aging Research, 4, 23-35.

Gosnell, B.A., Levine, A.S., & Morley, J.E. (1983). The effects of aging on opiod modulation of feeding in rats. Life Sciences, 32, 2793-2799.

Govoni, S., Loddo,, P., Spano, P.F., & Trabuchi, M. (1977). Dopamine receptor sensitivity in the brain and retina of rats during aging. Brain Research, 138, 565-570.

Govoni, S., Memor, M. Saiani, L., Spano, P.F., & Trabucchi, M. (1980). Impairment of Brain neurotransmitter receptors in aged

rats. Mechanisms of ageing and Development, 12, 39-46.

Greenberg, L.H., & Weiss, B. (1978). B-adrenergic receptors in aged rat brain: Reduced number and capacity of pineal gland to develop supersensitivty. Science, 201, 61-63.

Grossman, S.P., & Grossman, L. (1973). Persisting deficits in rats recovered from transections of fivers which enter or leave the hypothalamus laterally. Journal of Comparative and Physiological Psychology, 85, 515-527.

Hamm, R.J. (1981). Hypothermia-induced retrograde amnesia in mature and aged rats. Developmental Psychobiology, 14, 357-364.

Hamm, R.J., & Knisley, J.S. (1985). Environmentally-induced analgesia: an age-related decline in an endogenous opioid system. J. Geront., in review.

Hamm, R.J., Knisley, J.S., & Watson, A. (1985). Environmentally-induced analgesia: age-related changes in a hormonally-mediated, nonopioid system. Journal Geront., in review.

Hardy, J., Wolff, H. & Goodell, H. (1943). The pain threshold in man. Amer. J Psychiatr., 99, 744-751.

Harkins, S.W., & Chapman, C.R. (1977). The perception of induced dental pain in young and elderly women. Journal of Gerontology, 32, 428-435.

Hayes, R.L., Bennett, G.J., Newlon, P.G., & Mayer, D.J. (1976). Analgesic effects of certain noxious and stressful manipulations in the rat. Society for Neuroscience Abstracts, 2, 1350.

Hayes, R.L., Bennett, G.J., Newlon, P.G., & Mayer, D.J. (1978).

Behavioral and physiological studies of non-narcotic analgesia in the rat elicited by certain environmental stimuli. Brain Research, 155, 69-90.

Hess, G.D., Joseph, J.A., & Roth, G.S. (1981). Effect of age on sensitivity to pain and Brain opiate receptors. Neurobiology of Aging, 2, 49-55.

Hess, G.D., & Reigle, G.D. (1970). Adrencortical responsiveness to stress and ACTH in aging rats. Journal of Gerontology, 25, 354-358.

Hess, G.D., Riegle, G.D., & Frantz, W.L. (1970). Effects of chronic adrenocortical stimulation in the aging male rat. Fed. Proceed., 29, 452.

Hess, G.D., & Riegle, G.D. (1972).Effect of chronic ACTH stimulation on adrenocortical function in young and aged rats. American Journal of Physiology, 222, 1458-61.

Himsworth, R.L. (1970). Hypothalamic control of adrenaline secretion in response to insufficient glucose. Journal of Physiology, 198, 451-465.

Holoday J.W., Law, P.Y., Tseng, L.F., Loh, H.H., & Li, C.H.(1978). Beta-endorphin: pituitary and adrenal glands modulate its action. Proceed. of the National Acad. of Sciences, 74, 4628-4632.

Holoday, J.W., Law, P.Y., Loh, H.H., & Li, C.H. (1979). Adrenal steroids indirectly modulates opiate sensitivity. Journal of Pharmacol. and Experimental Therapeutics, 208, 176-183.

Hughes, J., Smith, T., Kosterlitz, H.W., Fothergill, L.A., & Morgan, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature, 258, 577-578.

Ida, Y., Tanaka, M, Tsuda, A., Kohno, Y., Hoaki, Y. , Nakagawa, R., Iimori, K., & Nagasaki, N. (1984). Recovery of stress-induced increases in noradrenaline turnover is delayed in specific brain regions of old rats. Life Sciences, 34, 2357-2363.

Ida, Y., Tanaka, M., Kohno, Y., Nakagawa, R., Iimori, K., Tsuda, A., Hoaki, Y., & Nagasaki, N. (1982). Effects of age and stress on regional noradrenaline metabolism in the rat brain. Neurobiology of Aging, 3, 233-236.

Jacquet, Y.F., & Lajtha, N. (1976). The periaqueductal grey: sites of morphine analgesia and tolerance as shown by 2-way cross tolerance between systemic and intracerebral injections. Brain Research, 103, 501-514.

Jensen, R.A., Martinez, J.L., McGaugh, J.L., Messing, R.B., & Vasquez, B.J. (1980). Psychobiology of aging. In G.J. Maletta and F.J. Pirozzolio (Eds.). The Aging Nervous System, NY: Praeger.

Jensen, R.A., Messing, R.B., Martinez, J.L., Vasquez, B.J., & McGaugh, J.L. (1980). Opiate modulation of learning and memory in the rat. In L.W. Poon (Ed) Aging in the 1980's, Washington D.C.: American Psych. Assoc., pp. 191-200.

Johannesson, T., & Becker, B.A. (1973). Morphine analgesia in rats

at various ages. Acta Pharmacol. et Toxicol., 33, 429-441.

Jonec, V., & Finch, C.E. (1975). Ageing and dopamine uptake by subcellular fractions of the C5713L/ J male mouse brain Brain Research, 91, 197-215.

Kanungo, M.S., Patnaik, S.E., & Koul,O. (1975). Decrease in 17B-estradiol receptors in brain of aging rats. Nature (London), 253, 366-367.

Kastin, A.J., Jemison, M.T., & Coy, D.H. (1979). Analgesia after peripheral administration of enkephalin and endorphin analogues. Pharmacology Biochemistry and Behavior, 11, 713-716.

Kato, R., Vassanelli, P., Frontino, G., & Chiesera, E. (1964). Variations in the activity of liver microsomal drug metabolizing enzymes in rats in relation to age. Biochem. Pharmacol., 13, 1037-1051.

Kavaliers, M., Hirst, M. & Teskey, G.C. (1983). Aging, opioid analgesia, and the pineal gland. Life Sciences, 32, 2279-87.

Kelly, D.D. (1982). The role of endorphins in stress-induced analgesia Annals of NY Academy of Sciences, 398, 260-271.

Kirchgessner, A.L., Bodnar, R.J., & Pasternak, G.W. (1982). Nalaxazone and pain- inhibitory systems: evidence for a collateral inhibition model. Pharmacology, Biochemistry and Behavior, 17, 1175-1179.

Kitahara, A., & Adelman, R.C. (1979). Altered regulation of insulin secretion in isolated islets of different sites in aging rats Biochem. Biophys. Res. Comm., 87, 1207-1213.

Klug, T.L., & Adelman, R.C. (1979). Altered hypothalamic-pituitary regulation of thyrotropin (TSH) in male rats during aging. Endocrinology, 104, 1136-1142.

Kumar, M.S.A., Chen, C.L., & Huang,H.H. (1980). Pituitary and hypothalamic concentration of Met-enkephalin in young and old rats. Neurobiology of Aging, 1, 153-155.

Kuhar, M.J., Pert, C.B., & Snyder, J.H. (1973). Regional distribution of opiate receptor binding in monkey and human brain. Nature, 245, 447-450.

Kuraishi, Y., Harada, Y., Aratini, S., Satoh, M., & Takagi, H. (1983). Seperate involvement of the spinal noradrenergic and serotonergic systems in morphine analgesia: the differences in mechanical and thermal algesic tests. Brain Research, 273, 245-52.

Lasagna, L. (1971). Influence of age on analgesic pain relief. J. Amer. Med. Assoc., 218, 1831.

Lippa, A.S., Pelham, R.W., Beer, B., Critchett, D.J., Dean, R.L., & Bartus, R.T. (1980). Brain cholinergic dysfunction and memory in aged rats. Neurobiology of Aging, 1, 13-19.

Lowy, M., Maickel, R.P., & Yim, G.K.W. (1980). Nalaxone reduction of stress-related feeding. Life Sciences, 26, 2113-2118.

Lytle, L.D., & Altar, A. (1979). Diet, central nervous system, and aging. Federation Proceedings, 38, 2017-2022.

Maggi, A., Schmidt, M.J., Ghetti, B., & Enna, S.J. (1979). Effect of aging on neurotransmitter receptor binding in rat and human

brain. Life Sciences, 24, 367-74.

Makman, M.H., Ann, H.S., Thal, L.J., Sharpless, N.S., Dvokrin, B., Horowitz, S.G., & Rosenfeld, M. (1979). Aging and monoamine receptors in brain Federation Proceedings, 37, 1922-26.

Makman, M.H., Ahn, H.S., Thal, L.J., Sharpless, N.S., Dvorkin, B., Horowtiz, S.C., & Rosenfeld, M. (1980). Brain Research, 192, 177-83.

Marek, P., Ponocka, I., & Hartmann, G. (1982). Enhancement of stress-induced analgesia in adrenalectomized mice: its reversal by dexmethasone. Pharmacology, Biochemistry and Behavior, 16, 403-405.

Mayer, D.J., & Hayes, R.L. (1975). Stimulation-produced analgesia: development of tolerance and cross tolerance to morphine. Science, 188, 941-943.

Mayer, D.J., & Price, D.D. (1976). Central nervous system mechanisms of analgesia. Pain, 2, 379-404.

Mayer, D.J., Wolfe, T.L., Akil, H., Carder, B., & Liebskind, J.C. (1971). Analgesia from electrical stimulation in the brainstem of the rat. Science, 174, 1351-1354.

McCarty, R. (1981). Aged rats: Diminished sympathetic-adrenal medullary responses to acute stress. Behavioral and Neural Biology, 33, 204-212.

McDougal, J.N., Marques, P.R., & Burks, T.F. (1980). Age-related changes in Body temperature responses to morphine in rats. Life Sciences, 27, 2679-2685.

McDougal, J.N., Marques, P.R., & Burks, T.F. (1981). Reduced tolerance to morphine thermoregulatory effects in senescent rats. Life Sciences, 28, 137-145.

McGeer, E.G., Fibiger, H.C., McGeer, P.L., & Wickson, V. (1971). Aging and brain enzymes. Experimental Gerontology, 6, 391-396.

Meek, J.L., Bertilsson, L., Cheney, D.L., Zsilla, G., & Costa, E. (1977). Aging induced changes in acetylcholine and serotonin content of discrete rat brain nuclei. Journal of Gerontology, 32, 129-131.

Messing, R.B., & Lytle, L.D. (1977). Serotonin-containing neurons: their possible role in pain and analgesia. Pain, 4, 1-21.

Messing, R.B., Vasquez, B.J., Samaniego, B., Jensen, R.A., Martinez, J.L., & Mcgaugh, J.L. (1981). Alterations in Dihydromorphine binding in cerebral hemispheres of aged male rats. Journal Neurochemistry, 36, 784-90.

Messing, R.B., Vasquez, B.J., Spiehler, V.R., Martinez, J.L., Jensen, R.A., Rigter, J., & McGaugh, J.L. (1980). Dihydromorphine binding in brain regions of young and aged rats. Life Sciences, 26, 921-27.

Miller, A.E., & Riegle, C.D. (1975). Aging effects on hypothalamic catecholamines and testosterone in the male rat. Federation Proceedings, 34, 303-309.

Miller, A.E., Shaar, C., & Riegle, G. (1976). Aging Effects on hypothalamic dopamine and norepinephrine content in the male rat. Experimental Aging Research, 2, 475-480.

Misra, C.H., Shelat, H.S., & Smith, R.C. (1980). Effects of age on adrenergic and dopaminergic receptor binding in rat brain. Life Science, 27, 521-526.

Moudgil, V.K., & Kanungo, M.S. (1973). Effect of age of the rat on induction of acetylcholinesterase of the brain by 17B-estradiol. Biochim. Biophys. Acta, 329, 211-220.

Mousa, S., Miller, C.H., & Couri, D. (1983). Dexamethasone and stress-induced analgesia. Psychopharmacology, 79, 199-202.

Nicak, A. (1971). Changes of sensitivity to pain in relation to postnatal development in rats. Experimental Gerontology, 6, 111-114.

Oliveras, J.L., Besson, J.M., Guilbaud, G., & Liebeskind, J.C. (1974). Behavioral and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. Experimental Brain Research, 20, 30-44.

Oliveras, J.L., Hosobuchi, Y, Redjemi, F., Guilbaud, G., & Besson, J.M. (1977). Opiate antagonist, nalaxone, strongly reduces analgesia induced by stimulation of raphe nucleus (centralis inferior). Brain Research, 120, 221-229.

O'Sullivan, J.B., Mahan, C.M., Freedlender, A.E., & Williams, A.F. (1971). Effect of age on carbohydrate metabolism. Journal of Clinical Endocrinology and Metabolism, 33, 619-23.

Pare, W.P. (1969). Age, Sex, and strain differences in the aversive threshold to grid shock in the rat. Journal of Comparative and Physiological Psychology, 69, 214-18.

Pasternak, G.W., Childers, S.R., & Snyder, S.H. (1980). Opiate anaglesia: evidence for mediation by a subpopulation of opiate receptors. Science, 208, 514-516.

Pedigo, N.W., Dewey, W.I., & Harris, L.S. (1975). Determinants and characteristics of the antinociceptive activity of IV administered acetylcholine in mice. Pharmacology and Experimental Therapeutics, 193, 845-852.

Peng, M.T., & Huang, H.H. (1973). Aging of hypothalamic-pituitary-ovarian furnction in the rat. Fertil. Steril., 23, 535-542.

Pert, A., & Bowie, D.L. (1979). Behavioral manipulations of rats causes alterations in opiate receptor occupancy. In E. Usdin, W.E. Bunney, N.S. Kline (Eds.) Endorphins in Mental Health Research, NY: Oxford, pp. 93-104.

Pert, A., & Walter, M. (1976). Comparison between nalaxone reversal of morphine and electrical stimulation induced analgesia in the rat mesencephalon. Life Sciences, 19, 1023-1032.

Pert, C.B., Pert, A., Chang, J.K., & Fong, B.J.W. (1976). D-Ala-enkephalamide: a long lasting synthetic peptide antagonist. Science, 194, 330-332.

Pert, C.B., Snyder, S.H. (1973). Opiate receptor: demonstration in nervous tissue. Science, 179, 1011-1014.

Pfeifer, W.D., & Davis, L.C. (1975). Modification of the adrenal stress response by age and prior experience. Exp. Aging Res., 2, 27-36.

Ponzio, F., Brunello, N., & Algeri, S. (1978). Catecholamine synthesis in brain of aging rats. Journal Neurochemistry, 30, 1617-1620.

Proudfit, H.K., & Anderson, E.G. (1975). Morphine analgesia: blockade by raphe magnus lesions. Brain Research, 98, 612-618.

Reaven, E.P., Gold, G., & Reaven, G.M. (1979). Effect of age on glucose-stimulated insulin release by the beta-cell of the rat. J. Clin. Invest., 64, 591-597.

Reis, D.J., Ross, R.A., & Joh, T.H. (1977). Changes in the activity and amounts of enzymes synthesizing catecholamines and acetylcholine in brain, adrenal medulla, and sympathetic ganglia of aged rat and mouse. Brain Research, 136, 465-474.

Rhodes, P.L. (1979). Periventricular system lesions in stimulation-produced analgesia. Pain, 7, 31-51.

Riegle, G.D., Meites, J., Miller, A.E., & Wood, S.M. (1979). Effect of aging on Hypothalmic LH-releasing and prolactin inhibiting activities and pituitary responsiveness to LHRH in the male laboratory rat. Journal of Gerontology, 11, 13-18.

Riegle, G.D., & Miller, A.E. (1978). Aging effects on the hypothalamo-hypophyseal-gonadal control system in the rat In E.L. Schneider (Ed). The Aging Reproductive System, NY: Raven Press, pp. 159-192.

Ritter, S., Bellin, S.I., & Pelzer, N.L. (1981). The role of gustatory and postingestive signals in the termination of delayed glucoprivic feeding and hypothalamic norepinephrine

turnover. J. of Neuroscience, 1, 1354-1360.

Ritter, R.C., & Neville, M. (1976). Hypothalamic noradrenaline turnover is increased during glucoprivic feeding. Fed. Proceed., 35, 642-647.

Ritter, S., & Pelzer, N.L. (1978). Magnitude of stress-induced brain norepinephrine depletion varies with age. Brain Research, 152, 170-175.

Ritter, S., Pelzer, N.L., & Ritter, R.C. (1978). Absence of glucoprivic feeding after stress suggests impairmentof noradrenergic neuron function. Brain Research, 149, 399-411.

Ritter, R.C., Roelke, M., & Neville, M. (1978). Glucoprivic feeding behavior in absence of other signs of glucoprivation. American Journal of Physiology, 234, E617-E621.

Ritter, S., & Ritter, R.C. (1977). Protection against stress-induced brain norepinephrine depletion after repeated 2-Deoxy-D-glucose administrations. Brain Research, 127, 79-84.

Rodeck, H., Lederis, K., & Heller, H. (1960). The hypothalamo-neurohypophyseal system in old rats. Journal Endocrinology, 21, 225-28.

Roth, G.S. (1974). Age related changes in specific glucocorticoid binding by steroid responsive tissues of rats. Endocrinology, 94, 82-90.

Roth, G.S. (1976). Reduced glucocorticoid binding site concentrations in cortical neuronal perikarya from senescent rats. Brain Research, 107, 345-54.

Roth, G.S. (1979). Hormone receptor changes during adulthood and senescence: significance for aging research. Fed. Proceed., 38, 1910-14.

Samanin, R., Ghezzi, D., Mauron, C., & Valzelli, L. (1973). Effect of midbrain raphe lesions on the antinociceptive action of morphine and other analgesics in rats. Psychopharmacology, 33, 365-368.

Samanin, R., & Valzelli, L. (1971). Increase of morphine-induced analgesia by stimulation of the nucleus raphe dorsalis. European Journal of Pharmacology, 16, 298-302.

Samorajski, T., Rolsten, C., & Ordy, J.M. (1971). Changes in behavior, brain and neuroendocrine chemistry with age and stress in C57BL/10 male mice. Journal of Gerontology, 26, 168-175.

Sartin, J., Chaudhuri, M., Obenrader, M., & Adelman, R.C. (1980). The role of hormones in changing adaptive mechanisms during aging. Federation Proceedings, 39, 3163-3167.

Sartin, J.L., Pritchett, J.F., & Marple, D.N. (1977). TSH, theophylline and cyclic AMP: in vitro thyroid activity in aging rats. Mol. Cell. Endocrinol., 9, 215-222.

Saunders, D.R., Paolino, R.M., Bousquet, W.F., & Miya, T.S. (1974). Age-related responsivenss of the rat to drugs affecting the central nervous system. Proceed. Soc. Experimental Biology and Medicine, 147, 593-595.

Sawynok, J., Pinsky, C., & LaBelle, F.S. (1979). On the specificity of nalaxone as an opiate antagonist. Life Sciences, 25,

1621-1632.

Schmidt, M.J., & Thornberry, J.F. (1978). Cyclic AMP and cylic GMP accumulation in vitro in brain regions of young, old and aged rats. Brain Research, 139, 159-177.

Schluderman, E., & Zubek, J.P. (1962). Effect of age on pain sensitivity. Perceptual and Motor Skills, 14, 295-301.

Segal, P.E., & Timiras, P.S. (1975). Age-related changes in thermoregulatory capacity of tryptophan deficient rats. Fed. Proceed., 34, 49-51.

Shaar, C.J., Euker, J.S., Riegle, G.D., & Meites, J. (1975). Effects of castration and gonadal steroids on serum and prolactin in old and young rats. Journal of Endrocrinology, 66, 45-51.

Sherman, E.D., & Robillard, E. (1960). Sensitivity of pain the aged. Canadian Med. Assoc. Journal, 83, 944-947.

Simone, D.A., & Bodnar, R.J. (1982). Modulation of antinociceptive responses following tail pinch stress. Life Sciences, 30, 719-729.

Simpkins, J.W., Mueller, G.P., Huang, H.H., & Meites, J. (1977). Evidence for depressed catecholamines and enhanced metabolism in aging female rats: possible relation to gonadotropin secretion, Endocrinology, 100, 1672-78.

Sladek, C.D., McNeill, T.H., Gregg, C.M., Blair, M.L., & Baggs, R.b. (1981). Vasopressin and renin response to dehydration in aged rats. Neurobiology of Aging, 2, 293-302.

Smith, G.P., & Epstein, A. (1969). Increased feeding in response

to decreased glucose utilization in the rat and monkey. Amer. J. Physiol., 217, 1083- 1087.

Spiaggia, A., Bodnar, R.J., Kelly, D.D., & Glusman, M. (1979). Opiate and non-opiate mechanisms of stress-induced analgesia:cross-tolerance between stressors. Pharm., Biochem. and Behavior, 10, 761-765.

Spratto, A., Dorio, R.E. (1978). Effect of age on acute morphine response in the rat. Research Commun. Chem. Pathology and Pharmacology, 19, 23-26.

Stratmentinoli, G., Gualano, M., Catto, E., & Algeri, S. (1977). Tissue levels of S- adenosylmethionine in aging rats. J. of Geront., 32, 392-394.

Stricker, E.M., & Zigmond, M.J. (1974). Effects of homeostasis on intraventricular injection of 6-hydroxy-dopamine in rats. Journal Comp.& Physiol. Psych. 86, 973-994.

Sun, A.Y. (1976). Aging and in vivo norepinephrine-uptake in mammalian brain. Experimental Aging Research, 2, 207-19.

Takemori, A.E., Tulunay, F.C., & Yano, I. (1975). Differential effects on morphine analgesia amd antagonism by biogenic amine modulators. Life Sciences, 7, 21-28.

Tang, F., & Phillips, J.G. (1978). Some age-related changes in pituitary adrenal function in the male laboratory rat. J. Gerontology, 33, 377-382.

Tenen, L.S. (1968). Antagonism of the analgesic effectof morphine and other drugs by p-chlorophenylalanine, a serotonin depleter.

Psychopharmacology, 12, 278-285.

Terman, G.W., Shavit, Y., Lewis, J.W., Cannon, J.T., & Liebeskind, J.C. (1984). Intrinsic mechanisms of pain inhibition: activation by stress. Science, 226, 1270-1277.

Thurmond, J.B., & Heishman, S.J. (1984). Effect of catecholamine precursors on stress- induced changes in motor activity, exploration and brain monoamines in young and aged mice. Behavioral Neuroscience, 98, 506-517.

Timiras, P.S. (1978). Biological perspectives on aging. Amer. Sci., 66, 605-613.

Timiras, P.S., & Hudson, D.B. (1980). Changes in neurohumoral transmission during aging of the central nervous system, In R.C. Adelman, J. Roberts, G.T. Baker,III, S.I. Baskin, and V.J. Cristofalo (Eds.) Neural Regulatory Mechanisms During Aging, NY: Alan R. Liss, Inc., pp 25-51.

Turkington, M.R., & Everitt, A.V. (1976). The neurohypophysis and aging with special reference to the antidiuretic hormone. In A.V. Everitt and J.A. Burgess (Eds.), Hypothalamus, Pituitary and Aging, Springfield, IL.: C.C. Thomas, pp. 123-136.

Vijayan, V.K. (1977). Cholinergic enzymes in the cerebellum and hippocampus of the senescent mouse. Experimental Gerontology, 12, 7-11.

Wallace, M.M., Bodnar, R.J., Kordower, J.H., Nilaver, G., & Zimmerman, E.A. (1980). Neuropeptidergic modulation of pain perception. Proceed. Amer. Pain Soc. 2, 19.

Watkins, W.B., & Choy, V.J. (1980). The impact of aging on neuronal morphology in the rat hypothalamo-neurohypophysial system: an immunohistochemical study. Peptides, 1, suppl 1, 239-245.

Watkins, L.R., & Mayer, D.J. (1982). The neural organization of endogenous opiate and non-opiate pain control systems. Science, 216, 1185-1192.

Webster, G.W., Shuster, L., & Eleftheriou, B.E. (1976). Morphine analgesia in mice of different ages. Experimental Aging Research, 2, 221-23.

Welsh, K.A., & Gold, P.E. (1984). Age-related changes in brain catecholamine responses to a single footshock. Neurobiol. Aging, 5, 55-59.

Wick, A.M., Drury, D.R., Nakada, H.I., & Wolfe, J.B. (1957). Localization of the primary metabolic block produced by 2-deoxy-D-glucose. Journal of Biological Chemistry, 224, 963-979.

Yaksh, T.L. (1981). Spinal opiate analgesia: Characterization and principles of action, Pain, 11, 293-346.

Yaksh, T.L., & Rudy, T.A. (1978). Narcotic analgetics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. Pain, 4, 299-359.

Yaksh, T.L., Yeung, J.C., & Rudy, T.A. (1976). An inability to antagonize with nalaxone the elevated nociceptive thresholds resulting from electrical stimulation of the mesencephalic grey. Life Sciences, 18, 1193-98.

Zbuzek, B.k., & Wu, W. (1982). Age-related vasopressin changes in

rat plasma and the hypothalamo-neurohypophyseal system Exp. Gerontology, 17, 133-138.