MINIREVIEW

The Dorsomedial Hypothalamic Nucleus Revisited: 1998 Update (44296)

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Abstract. **This article reviews data that have accumulated since the early 1970s on the role of the dorsomedial hypothalamic nucleus (DMN) in neuroendocrine and autonomic homeostasis. Both the ventromedial hypothalamic nucleus (VMN) and the lateral hypothalamic area (LHA) project to the DMN, which in turn projects to the paraventricular nucleus of the hypothalamus (PVN), thus placing the DMN at an important nodal point of neuroendocrine/autonomic circuitries. The DMN is composed of cells and fibers containing neuropeptide Y (NPY), and the nutritional status (starvation-refeeding) is reflected in NPY levels of both VMN and DMN in Sprague-Dawley, Zucker (*fa/fa*), and corpulent rats (*cp/cp* JCR:LA). The DMN is involved in the final common pathway of corticotrophin-releasing hormone (CRH) secretion by the PVN, sympathetic nervous system outflow to the adrenal gland, and brown adipose tissue (BAT) thermogenesis. The DMN is also part of a "fear circuitry" regulating cardiovascular responses to stress such as myocardial blood flow and the tachycardia associated with the defense reaction. This appears to be mediated by a gamma amino butyric acid (GABA) mechanism. Although exhibiting reduced ponderal and linear growth and hypophagia and hypodipsia, the rat with DMN lesions (DMNL rat) has normal body composition, anabolic hormone levels, and intermediary metabolism, and it responds normally to numerous endocrine, nutritional, intra- and extracellular thirst and body weight–regulatory challenges. The DMNL rat shows normal efficiency of food utilization, but shows an attenuated response to the feeding-stimulatory effect of insulin. The only other lesion-induced abnormalities are hyperprolactinemia and a disrupted circadian corticosterone rhythm. The hyperprolactinemia in DMNL rats appears to be related to an attenuation of dopamine (DA). Rats with DMNL are capable of mating and can bear offspring, but there is a dramatic effect on litter size and other litter parameters that only improves when one parent is a DMNL rat. Antiaging effects produced by DMNL are evident in the prevention of age-associated microalbuminuria and kidney lesions, as well as, in prevention of the age-related decline in circulating insulin-like growth factor I (IGF-I). Recent evidence suggests that DMN, together with the VMN and the arcuate nucleus (ARC) of the hypothalamus, may be part of the circuitry that is responsive to the feedback signal from adipose tissue by the hormone leptin. The above findings and others suggest that the DMN plays a diverse role in physiological regulatory processes.** [P.S.E.B.M. 1998, Vol 218]

The role of such "classical" hypothalamic feeding and body weight–regulatory areas as the VMN (1–6) and the LHA in neuroendocrine and neuroautonomic homeostasis has been investigated extensively since the early 1940s, yet studies in the role of other hypothalamic areas, such as the PVN (7, 8) and the DMN (9–11) did not

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begin until much later. The early investigations of the DMN [reviewed in the mid-1970s (10)] and late '80s (11) concentrated primarily on the role of this nucleus in feeding, drinking, body weight regulation, and their underlying mechanisms.

During the last decade, numerous investigators from many areas of research in physiology have directed their attention to a variety of additional direct or indirect functions of the DMN (12–19). These recent findings provide a more mature look, a Gestalt view, at the functions of the DMN.

The present review article is an attempt to fit recent findings into a larger picture since the DMN was last reviewed 10 years ago. For space reasons the reader will be referred frequently to the earlier review articles for expanded coverage, detailed background information, and references.

Neuroanatomy, Connectivity

The neuroanatomy of the DMN and its connectivity were extensively covered in our earlier review (11) and will only be summarized briefly here. Direct and indirect connections have been demonstrated between the DMN and both the sympathetic and the parasympathetic moieties of the autonomic nervous system. Notably, the DMN also has direct connections to and from the nucleus tractus solitarii (NTS), the parabrachial nucleus, and the sympathetic intermediolateral columns that bring the DMN under the influence of peripheral afferents.

Connections from the DMN have also been traced to the mesencephalon and the origin of the hypothalamoreticular tract. This provides a pathway by which the DMN may influence the reticular formation and therefore the general arousal system. The DMN also connects to the precentral motor cortex and sensorimotor areas, the hippocampus, the PVN and the suprachiasmatic nucleus (SCN). These connections bring the DMN in direct contact with circuits of both autonomic and neuroendocrine significance and the principal circadian clock, respectively. The DMN is also connected to the posterior hypothalamus and extends projections to the cerebellum.

Regarding intrahypothalamic projections, the DMN has been reported to connect with both VMN and LHA, the "classical" hypothalamic feeding areas, thus providing a potential modulator role on the activities and the output from these two hypothalamic loci. Finally, there are connections between the DMN and the circumventricular organs (i.e., the area postrema (AP), organum vasculosum lamina terminalis (OVLT), and subfornical organ (SFO)) which bring it in contact with the blood brain barrier (11). However, the Swanson group (20) offers a discordant view: although they observed a few unbranched DMN fibers projecting to the SFO and an occasional fiber in the AP, they saw no fibers in the OVLT.

Findings by Petrovicky (21) indicated that most fibers from the medial hypothalamic area (i.e., the VMN and DMN) enter the LHA and thereafter course caudally through the medial forebrain bundle. "Hence any lesion involving the lateral hypothalamus is associated with fibers proceeding from the medial hypothalamus" (21). LHA lesions (L) may thus precipitate changes that are not so much due to LHA damage *per se,* but may be due partly to disrupted signal flow from the medial hypothalamic nuclei, such as VMN, DMN and PVN, or perhaps more likely, disturbed reverberant signal flow involving these three nuclei. Thompson *et al.* (20) more recently confirmed that many fibers leave the DMN laterally and travel through the LHA "where most of them display the appearance of fibers-of-passage."

Other authors (22) also reported neurons occupying the lateral parts of the DMN and VMN to extend their dendrites into the LHA (note their Fig. 3-9). Furthermore, some neurons around the fornix project dendrites to both the medial nuclei and LHA.

Using electron microscopic techniques, Zaborsky *et al.* (23) found that, following transsection at the level of the posterior hypothalamus-mamillary body in the rat, direct pathways could be traced from the site of transsection to the ARC, VMN, and DMN and even as far rostral as the PVN and supraoptic nucleus (SON). These pathways were also closely appositioned to the wall of the third ventricle, but deviated in lateral direction at the level of the DMN ". . . to which nucleus the tract issues preterminal fibers" (23). Terminal degeneration patterns indicated that the DMN is reached from posteriorly through the periventricular system, but that the degeneration was found to be concentrated in the lateral part of the DMN (23). Another fiber group was shown to reach the nucleus laterally, from the direction of the zona incerta (ZI).

Subsequent studies by Ter Horst and Luiten (24) using phaseolus vulgaris leucoagglutinin (PHAL) tracing, revealed connections between VMN, LHA, and DMN with the PVN in the rat. Whereas the previous consensus was that VMN and LHA both project to the DMN, and the latter might integrate this information before projecting it either to other parts of the central nervous system (CNS) or directly to the periphery, it was now established that ". . . the main projection of the DMN is aimed at the parvocellular paraventricular nucleus." Notably, the DMN was shown to contribute only small numbers of projections to LHA and VMN, whereas tracer deposits in LHA and VMN resulted in anterograde labeling of the DMN. This finding was recently confirmed by Thompson *et al.* (20). Quite remarkably, the PVN gives rise to only minor reciprocal projections to LHA, VMN, and DMN. Ter Horst and Luiten (24) concluded from their work that ". . . the main stream of connections in the hypothalamus runs from the LHA and VMH to the DMH and from the DMH to the PVN." With such connections, the DMN could modulate/integrate the activities of both VMN and LHA in the regulation of pancreatic hormones and metabolic processes *via* both the neuroautonomic and the neuroendocrine systems (25). In general, Thompson *et al.* (20) agreed with this view and added that the DMN also

significantly innervates the forniceal and lateral parvicellular parts of the PVN. These projections have obvious functional significance as these parvicellular parts of the PVN project densely to autonomic and premotor nuclei of the brainstem and spinal cord.

More recent PHAL anterograde tract-tracing studies by Wagner *et al.* (26) revealed that the ZI, considered a rostral extension of the mesencephalic reticular formation (27), sends efferent DA-ergic fibers ipsilaterally to a variety of brain regions. Within the hypothalamus, the heaviest labeling was seen in the anterior hypothalamic area (AHA), LHA and parvocellular PVN, moderate labeling was observed in the contralateral parvocellular PVN, and light labeling was seen in SON, ARC, VMN and contralateral LHA. However, despite its immediate proximity to the ZI, the DMN was only lightly labeled. The heavy labeling in the aforementioned areas may be understood in view of the role that the ZI plays in the integration of complex somatosensory behaviors (28).

In addition to the commonly used laboratory rat, anatomical investigations of the DMN have also been reported in the pig (29), the hedgehog, mole, the common shrew (30), and more recently, the lizard *Gecko gecko* (31). In the gecko, wheat germ agglutinin conjugated to horse radish peroxidase and microinjected into the DMN labeled afferent neurons in the rostral and caudolateral poles of the dorsal cortex, anterior septal nucleus, horizontal limb of the diagonal band, nucleus of the anterior sommissure, several hypothalamic areas, the lateral habenula, the posteroventral thalamic nucleus, and cells scattered around the dorsolateral anterior thalamic nuclei. Labeled brainstem populations included parvocellular and ventral isthmal nuclei and the lateral dorsal tegmental nucleus. The authors compared these findings with those in amphibians and mammals (31).

In rodents, three cortical areas have been identified that project to the hypothalamus: the infralimbic area projects to the DMN and LHA (32), the agranular insular projects to the LHA, and the ventral subiculum projects to several hypothalamic areas, including the medial preptic area (mPOA), the AHA, shell of the VMN, DMN, ventral premamillary nucleus, and the area around the medial mamillary nucleus (33). The projection from the infralimbic area to the DMN and LHA of rodents appears most similar to the projections from the rostral lobe of the dorsal cortex to the DMN and LHA of geckos.

Fibers from the SCN undoubtedly play a role in DMN function. We reported earlier (34) that plasma circadian corticosterone rhythms are disrupted in the DMNL rat. This was thought to be due not to destruction of intrinsic Zeitgeber neurons in the DMN, but rather to interruption of fibers from the SCN to the DMN, bringing the latter under the influence of the former. More recently, such connections have been investigated by Kalsbeek *et al.* (35). Using the anterograde agent PHAL, they identified efferent projections from the SCN of the golden hamster (*Mesocricetus auratus*) and aided further localization by restricted SCN lesions and immunochemistry. First, major terminal fields of SCN-derived vasopressin (VP) were seen in the medial part of the DMN, mPOA, anterior paraventricular nucleus of the thalamus, and the medial parvocellular part of the hypothalamic PVN. Secondly, the medial DMN received vasoactive intestinal peptide (VIP) projections from the SCN, which were also seen in the thalamic paraventricular nucleus and the anterior and dorsal parvocellular hypothalamic PVN and the subparaventricular area. Lastly, the DMN received gastrin-releasing peptide (GRP) projections from the SCN. GRP projections were also found in the subparaventricular area. The DMN, SON, and subparaventricular area also contained GRP efferent fibers from the SCN. It was also noted that the contralateral SON was heavily innervated, a pathway for which the neurotransmitter had not been identified. These data show that three different neuronal populations of the SCN exhibit a clear diversity in their preferential target site, yet the DMN is supplied by all three (35). In a subsequent study, Thompson (20) found dense projections from the DMN to the peri-SCN regions with minor input to the dorsal DMN. In agreement with this finding, Moga and Moore (36) more recently found retrogradely labeled neurons in the DMN after injections of Fluoro Gold into the SCN and peri-SCN region. Notably, Merchenthaler *et al.* (37) reported numerous galanin-immunoreactive fibers within the SCN and peri-SCN; the DMN contains many galaninin-immunoreactive neurons (36). These findings make more likely our earlier hypothesis that the DMNL-induced circadian rhythm disruption for corticosterone was due to the disruption of SCN fibers projecting to the DMN (34).

In the human hypothalamus, Dai *et al.* (38) described that VP and VIP fibers arising from the SCN branch extensively and innervate the SCN itself and the DMN, as well as the anteroventral hypothalamic area, the area below the PVN, and the ventral part of the PVN. There appeared to be substantial congruity between the presumptive human SCN projections and those observed by tracing in rat or hamster (38).

In an immunohistochemical investigation in the neuroanatomical distribution of arginine-vasotocin-like systems in the roughskin newt (*Taricha granulosa*), Lowry *et al.* (39) noticed vasotocin-like cell bodies among others in the DMN. They were thought to be homologous to groups of VP-immunoreactive neuronal cell bodies described in mammals. The authors noted that the distribution of arginine-vasotocin-like systems in *T. granulosa* is greater than the distribution previously reported in any other single vertebrate species (39).

Studying local excitatory synaptic inputs to neurons of the PVN and SON, Boudaba *et al.* (40) conducted whole-cell voltage-clamp and current clamp recordings in the rat. The local excitatory inputs to PVN and SON are glutamate-mediated, as excitatory post-synaptic potentials/excitatory

postsynaptic currents elicited with electrical stimulation or metabotropic receptor activation (glutamate receptor agonists) were blocked by ionotropic glutamate receptor antagonists. Two sites that provided most of the local excitatory inputs to PVN neurons were the DMN and the perifornical region. This may play a role in pulsatile hormone release (40).

Berk and Finkelstein (41), using horseradish peroxidase, demonstrated efferent connections from the periequiductal gray (PAG) to the mPOA and the hypothalamus, including the DMN and PVN. In the above-quoted work by Ter Horst and Luiten (24) it was also shown that the majority of descending pathways from the DMN course dorsally from the DMN and proceed medially to the thalamus. From the dorsal diencephalic level, they take a caudal direction to reach the PAG. In the midbrain, the descending pathway continues to course through the PAG and then to the locus coeruleus (LC). It was shown (42) using single-unit recording in the DMN to obtain antidromic responses to stimulation of the LC that the majority of neurons of the DMN projecting to the LC were not spontaneously active. However, those projecting from the DMN to the median eminence tended to discharge spontaneously.

Using immunocytochemistry for proenkephalin, Beaulieu *et al.* (43) found a dense cluster of enkephalin-immunoreactive (IR) cell bodies within the DMN and the dorsal part of the parvicellular subdivision of the PVN. Additionally, retrograde transport of wheat germ–conjugated gold particles from the PVN, combined with immunohistochemistry for enkephalin, also revealed that a major source of extrahypothalamic enkephalin afferents to the PVN are provided by enkephalin neurons in the lateral reticular nucleus and the paragigantocellularis reticular nucleus of the medulla. Retrogradely labeled enkephalin neurons from the PVN were also found in the DMN, the mPOA, the lateral septum, and the ARC. The authors (43) suggest that these enkephalinergic pathways from the medulla and the forebrain represent an anatomical substrate that underlies the opioid effects on PVN neurons during cardiovascular regulation (the DMN is also involved in cardiovascular responses—see below), feeding, and stress response.

Transmitters

Serotonin/Dopamine. The DMN contains serotonin (5-HT) neurons that may be under DA-ergic and β-adrenergic receptor-mediated catecholaminergic influence (11). Using immunocytochemistry, Arezki *et al.* (44) microinjected intracerebroventricular (icv) ^{3}H-5-HT or ^{3}H-DA and found that all of the 5-HT-accumulating neurons (cell bodies, dentrites, and terminals) were distinct from the DA-labeled ones. In the DMN, only 5-HT-IR nerve cells could be detected after icv administration of 5-HT or DA. These cell bodies were distinctly different from the tyrosine hydroxylase-IR and DA-labeled neurons of the DA-ergic periventricular ARC complex. In the DMN, they could also observe an association between (^{3}H)-DA labeled and glutamate decarboxylase (GAD)-IR processes with 5-HT-IR or (^{3}H) 5-HT accumulating neurons.

The DMN has been implicated in an extrainfundibular DA system (11). Bjoerklund *et al.* (45) emphasized that whereas the tuberoinfundibular tract directly innervates the median eminence, their "incertohypothalamic dopamine system" (IHD) innervates the median eminence from a higher level and is an interdiencephalic system. Their lesion studies indicated that the system originates in the A11, A13, and A14 regions and is unaffected by lesions destroying the Ungerstedt (46) systems. Total removal of norepinephrine (NE) input from the lower brainstem did not affect the IHD system (45).

More recent findings indicate that cell bodies and terminals of this system are densely innervated by both NE and 5-HT neurons and that these systems may possibly interact, a notion we have previously entertained (11, 47). Inhibition of 5-HT neurons by the administration of the 5-HT autoreceptor agonist 2-hydroxy-2 (di-n-propylamino) tetralin (8-OH-DPAT) increased the accumulation of 3,4-dihydroxyphenylalanine (DOPA) in the DMN and the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) in both ZI and DMN, indicating an activation of catecholaminergic neurons in these two areas. Concentrations of 3-methoxy-4-hydroxyphenylethyleneglycol, a NE-metabolite, were increased in the ZI and DMN by 8-OH-DPAT or 5,7-dihydroxytryptamine-induced lesions of 5-HT neurons. This suggests that NE neurons terminating in these regions were activated following manipulations that decrease 5-HT neuronal function. Following the destruction of NE neurons projecting to the ZI and DMN, 8-OH-DPAT no longer increased DOPAC in both ZI and DMN. Evidently, 5-HT neurons inhibit the activities of NE neurons terminating in the ZI and the DMN, but they do not influence the activity of the IHD neurons (48).

Evidence for a differential regulation of 5-HT receptors in different brain regions concerned with feeding was recently provided by acute serotonergic denervation studies (49). Seven days after using 5,7-dihydroxytryptamine treatment, there was a significant increase in the density of 5-HT_{1a} receptors in both DMN and VMN. In adjacent sections, however, 5-HT_{1b} receptor density was significantly increased only in the VMN. Notably, there was no change in the LHA or hippocampus. Since binding of (^{3}-H) paroxetine, which labels presynaptic transported sites, was significantly decreased in all evaluated brain areas, it was concluded that in these brain regions 5-HT_{1a} and 5-HT_{1b} receptors are primarily postsynaptic (49).

Since the 5HT receptor 5-HT_{2C} may be involved in depression (50), the effect of the antidepressant imipramine (20 mg/kg orally for 4 days) was investigated in the rat. Hybridization signals in nearly all regions stained by digoxingenin-labeled antisense cRNA probe included the hippocampus, choroid plexus, habenular nucleus, and DMN.

These data show that antidepressants do indeed act as acute 5-HT_{2C} antagonists. The long-term occupation of 5-HT_{2C} receptor by antidepressants may stimulate 5-HT_{2C} receptor gene expression to compensate for the receptor function (51), the role of the DMN in the process awaits further study.

Neuropeptide Y (NPY). The DMN contains scattered cells and fibers of NPY. This peptide is the most potent food intake-stimulatory compound tested to date (52). The ARC contains a high concentration (57.8 ± 3.8 pmol/mg protein) of NPY cell bodies and pathways. The ARC NPY pathways (53) are projected into various areas of the hypothalamus including the DMN and PVN. High concentrations of NPY are also found in the PVN (57.9 ± 6.9 pmol/mg protein). The DMN contains 37.9 ± 8.0 pmol/mg protein, whereas the LHA contains much less NPY (10.0 ± 2.1 pmol/mg protein). Lesions in the ARC are followed by a decrease of NPY-containing fibers in both DMN and PVN (54).

Sahu *et al* (55) reported that the feeding status of the animal influences NPY levels. Food deprivation of 2, 3, and 4 days followed by 1 day of *ad lib* food intake in male rats showed that NPY-like-IR was unaffected by food deprivation and refeeding in the DMN, VMN, and median eminence. However, ARC, mPOA, and PVN displayed an entirely different pattern. In the ARC, NPY levels rose significantly after 3 and 4 days of deprivation and remained elevated even after 1 day of refeeding. In the mPOA, NPY levels did not change after 4 days of food deprivation but did increase after refeeding. In contrast, in the PVN, food deprivation elicited a gradual increase in NPY levels that reached a maximum concentration after 4 days. Following 1 day of refeeding, NPY levels dropped dramatically to the range found in satiated control rats.

More recently, Beck *et al.* (56) reported the feeding status of Sprague-Dawley rats influenced NPY levels in several hypothalamic nuclei. He and his group demonstrated that a 48-hr food deprivation resulted in a 5-fold increase in NPY in the PVN and a 10-fold increase in the ARC-median eminence area. Subsequent refeeding for 6 hr reversed the effect of food deprivation in the ARC-median eminence area, but the levels of NPY remained elevated in the PVN. Notably, the perifornical lateral hypothalamic area showed a significant rise in NPY content in refed rats as compared to either the satiated or deprived animals. The NPY levels in the DMN, VMN, SON, and SCN "... showed total resistance to any change following deprivation and feeding." These data emphasize the predominant role of the ARC and PVN in food deprivation-refeeding changes in NPY, whereas two other crucial hypothalamic areas involved in feeding (i.e., the VMN and the DMN) appear to be silent to these challenges (56).

Beck and his group (57) also investigated if regulation by the feeding state also occurs in the *fa/fa* rat. Groups of ten week-old lean and obese Zucker rats were: 1) fed *ad libitum;* 2) food-deprived for 48 hr; or 3) food-deprived for 48 hr and then refed for 6 hr. NPY concentrations in the ARC were about 50% greater in obese than in lean rats regardless of the feeding state. In the VMN, NPY concentrations were higher in the lean rats that were food deprived than in the obese, food-deprived rats. Food deprivation and refeeding did not modify NPY levels in the ARC, VMN, or DMN irrespective of genotype. However, food deprivation did induce a significant drop in NPY concentrations in the parvocellular region of the PVN of the lean rats. Notably, *ad lib* levels were restored after 6 hr of refeeding. These variations were not observed in the obese rat. Therefore, it was concluded that the regulation of NPY by the feeding state in the Zucker rat is very different from that described for lean SD rats (56). This might be explained by the genetic make-up or age differences of the animals used. The high NPY levels and absence of regulation in the obese Zucker rat could contribute to the abnormal feeding behavior of these animals (57). It is evident that the role of the DMN in this phenomenon is not crucial.

An investigation in freely fed obese and lean *fa/fa* Zucker rats by McKibbin *et al.,* (58), showed that concentrations of NPY as measured by radioimmunoassay were elevated in DMN, VMN, and PVN of obese rats when compared to lean counterparts. Notably, food restriction over 3 weeks that resulted in a weight loss of 25%, did not affect NPY levels in these hypothalamic areas. Refeeding in lean rats to the pre-reduction body weight caused an increase in NPY in both DMN and VMN but had no such effect in *fa/fa* animals. It was suggested that the behavioral and neuroendocrine changes seen in obese Zucker rats are the same as are observed after NPY injection (i.e., hyperinsulinemia, hyperphagia, hyperglucagonemia, hypercorticosteronemia, and elevated ACTH levels). It appeared that the DMN may be involved in the Zucker obese rat syndrome but is so only by a collaborative action of several hypothalamic nuclei known to be linked by an extensive neuronal network (24, 59). In a more recent study, the same group (60) reported that NPY concentrations in the ARC were similar in normophagic 16-day-old Zucker lean and obese pups. However, at 30 days of age, when the genetically obese rats had become hyperphagic, NPY concentrations were elevated compared to their lean counterparts. The NPY concentration was also increased at 30 days in LHA, DMN, and PVN, but there was no difference between genotypes (60).

A correlation between NPY concentrations and certain hypothalamic areas has also been established from investigations into another type of genetically obese rodents, the corpulent *cp/cp* JCR:LA rat (61). NPY levels were significantly (31%) higher in the ARC of these obese rats when compared to their lean counterparts. Food restriction in *cp/cp* rats brought about no change in ARC NPY, but it was elevated in the DMN (36%) and VMN (31%). Food restriction in lean rats, on the other hand, was followed by a 22% rise in ARC NPY, but a more dramatic rise (44%) in the DMN NPY. Evidently, a disregulation of NPY leading to higher levels of NPY in the ARC—its principal point of

synthesis—contributes to the intrinsic obesity and the accompanying hyperinsulinemia in the *cp/cp* rat (61). The DMN apparently plays a role in the refeeding process of this genetic model.

Since pharmacological antagonism of hypothalamic melanocortin receptors or genetic deletion of the melanocortin-4 receptor (MCR-4) suggests that chronic disruption of central melatoninergic signaling is the cause of the agouty-induced obesity, Kestersen *et al.* (62) performed *in situ* hybridization using an antisense NPY probe. Although the arcuate nucleus NPY mRNA levels were equivalent in obese A^y mice and their C57BL/6JJ litter mates, the DMN notably expressed high NPY levels above background only in obese C57BL/6J-A^y mice, which was highly significant when compared with C57BL/6J control mice ($P < 0.0001$). Expression of NPY was also seen in the DMN of obese Mc4-RKO (melanocortin-4 receptor knockout) homozygous (–/–) mice, but not in lean heterozygous or wild (+/+) type control mice. This identifies the DMN as a brain region that is functionally altered by the disruption of melanocortinergic signaling and suggests that the DMN, possibly *via* elevated NPY expression, may have an etiological role in the melanocortinergic obesity syndrome (62).

A further involvement of the DMN (and PVN and mPOA) in NPY action, is indicated by the demonstration that neuronal transection at the level of the dorsal tegmental area of the mesencephalon rendered rats hyperresponsive to NPY (63). Notably, NPY levels in the SCN, ARC, and VMN were not altered by neural transection, suggesting that NPY innervation in these areas may be derived mainly from NPY perikarya in the ARC and elsewhere in the diencephalon. However, NPY concentrations were markedly (50%–60%) decreased in the DMN, mPOA, PVN, and median eminence.

The DMN also plays a role in a neural network regulating food intake in the seasonal changes seen in hibernators such as the golden-mantled ground squirrel. The Woods group (64), using *in situ* hybridization, reported an abundant expression of NPY mRNA in the ARC of this animal whereas the mRNA expression for galanin (GAL), another peptide that stimulates feeding, was concentrated in both ARC and DMN. After intracerebroventricular (i.c.v.) injections of NPY (0.1, 0.5, 5.2, and 9 μg) or GAL (0.1, 0.5, 2.0, and 8 μg), food intake was significantly increased over the following 30 min in the case of GAL, and over 2 hr in the case of NPY. Evidently, both nonhibernators and hibernators share common neural substrates for the regulation of energy intake. Seasonal modulation of these neural networks may contribute to the annual cycles of food intake seen in hibernators (64).

Although the PVN and DMN are linked by connections that were described earlier by Conrad and Pfaff (65) and more recently by the elegant studies of Ter Horst and Luiten (24, 59), the DMN does not appear to be involved in the PVN-associated GAL-containing system and the associated ingestion of fat that has been demonstrated by Leibowitz and her group (66). Hypothalamic NPY administration preferentially enhances the intake of carbohydrate in the same animals that consume fat in response to GAL administration (67). The pentapeptide enterogastrin, shown to reduce appetite specifically for fat (68), strongly attenuates the GAL feeding response, but has no effect on feeding induced by NPY (69).

Using analyses of peptide levels *via* radioimmunoassay and gene expression by *in situ* hybridization, Akabayashi *et al.* (70) also established a close association between GAL in the PVN, particularly in the midlateral part, and fat ingestion. Notably, intra-PVN injections of GAL, enhanced fat intake and resulted in hypoinsulinemia but did not affect ingestion of carbohydrates or protein (67). No increase in fat intake was detected after GAL was injected into the DMN or the mPOA. In addition, PVN injection of antisense oligonucleotide to GAL mRNA caused a dramatic decline in fat ingestion and body weight. One function of PVN GAL may be to inhibit postprandial insulin secretion to maintain blood glucose levels during the process of fat absorption.

Gamma-aminobutyric Acid (GABA). The neurotransmitter GABA is present in the hypothalamus in large amounts, as concluded from the presence of high concentrations of its biosynthetic enzyme, glutamic acid decarboxylase (GAD) (21). A good correlation between GABA and GAD concentrations has been reported (73), but they may not necessarily co-exist in the same cells.

After total deafferentation of the medial hypothalamus, GAD remained unchanged in the median eminence, but fell markedly in the VMN and ARC. In these two nuclei, a decrease in GAD also occurred following partial deafferentation from the lateral and posterior hypothalamus, but not from the AHA and the mPOA (72).

Both the DMN and the LHA have been reported to contain low amounts of GABA (71–72). On the other hand, in a study aimed at determining the regional distribution of GABA within the hypothalamus, as well as an elucidation of the role of GABA in feeding, Kimura and Kuryama (72a) found the highest amount of GABA in the LHA. Furthermore, more recent investigations by Okamura *et al.* (73) studied the distribution of GABA-ergic cell groups in the hypothalamus by means of the *in situ* hybridization technique using a cDNA probe for messenger RNA encoding GAD. Major GABA-ergic cells groups were observed in the DMN, tuberomamillary, ARC, SCN, mPOA, AHA, perifornical area, and LHA.

The most prominent GAD mRNA containing cell groups were found in the mPOA, AHA, and DMN (73). The GABA-ergic nature of these cell populations was further supported by detection of GAD and GABA-IR. In the DMN, weakly labeled neurons were seen throughout the anterior DMN whereas, at the coronal plane of the posterior part of the median eminence, a group of densely aggregated small cells appeared in the center of the DMN. Notably, the VMN was virtually devoid of labeled cells. The distribution of GAD-IR cells corresponded well with that of cDNA-

labeled cells. The authors concluded that the abundance of GABA-ergic cells indicates that GABA represents quantitatively the most important transmitter of hypothalamic neurons and that it may be involved in neuroendocrine and autonomic regulatory functions (73).

More recently still, the DMN, along AHA, posterior bed nucleus of the stria terminalis, and mPOA has been shown to contribute presynaptic, inhibitory fibers to the PVN in both the horizontal and the parasagittal plane. These presynaptic sites each contain GABA-producing neurons, based on *in situ* hybridization with a GAD riboprobe, and together form a three-dimensional ring around the PVN (74).

The close proximity of the PVN and the DMN could conceivably mask the consequences of manipulations performed in/on the DMN or vice versa. To elucidate possible masking of DMN manipulations regarding the stress response, Keim and Shekhar (75) have recently microinjected the GABA(A) antagonist bicucculine methyodide (BMI) (50 pmol/100 nl) into the DMN and anterior sites (i.e., closer to the PVN). Rats injected with this GABA antagonist showed significant increases in heart rate, blood pressure, and plasma levels of ACTH and corticosterone. Notably, injections at anterior sites (i.e., closer to the PVN) did not elicit significant increases in these parameters. It was concluded that a tonic GABA(A)-mediated inhibition system regulates a coordinated physiological response in the DMN and that this response is not due to a diffusion of the antagonist into the PVN.

Opioids. Opioids are known to play a role in the control of feeding behavior. Whereas previous reports indicated a role for the DMN in opioid mechanisms (75a), subsequent (11) and more recent reports have strengthened such a role. In an analysis of several hypothalamic areas in the *ob/ob* mouse, (76) using the micropunch method on fresh, unfixed brain slices, found a five-fold increase of beta-endorphin in the DMN and a two-fold increase in the VMN in *ob/ob* versus lean mice. Dynorphin levels were comparable in *ob/ob* and lean mice in the anterior, LHA, VMN, and PVN. However, a five-fold increase in dynorphin levels from that of the lean mouse may represent a defect that results in the hyperphagia and the glucoregulatory deficits that are seen in the *ob/ob* mouse (76). An involvement of the DMN in acupuncture-induced analgesia has been suggested by the findings of Ishibashi (77). Acupuncture in the "L point" (lung) has been found to be useful in the treatment of opioid-addicted patients (78), and it has also been reported to result in increased endorphin concentrations in the cerebrospinal fluid (CSF) (79) and to modulate feeding behavior (80). Microinjection of morphine into the DMN has been shown to produce analgesic effects (81). When the L-point in the rat was stimulated by electroacupuncture, ". . . the neurons in the lateral hypothalamus and the ventromedial hypothalamus did not respond . . ." but 14% of the neurons that did respond were located in the DMN (77). The author attributed the failure of LHA and VMN neurons to respond to electroacupuncture to the fact that no direct connections exist between the L-point and food intake regulatory "centers" located in these two areas. Notably, it has been previously established that auricular acupuncture ". . . has a clinical effect on the treatment of obese patients . . ." (77). The effects of acupuncture do not always involve the LHA, but such connections, directly or indirectly, may involve the DMN.

Indeed, more recent findings (82) showed that after electroacupuncture in the "Zusanli" and "Sanyinjiao" points, the rate of spontaneous discharges and the rate of duration of the pain-invoked discharges of the pain-excitatory units in the DMN were dramatically decreased; the spontaneous firing rate of pain-inhibitory units was increased, whereas their inhibitory responses to nociceptive stimulation were released by the acupuncture. Evidently, the DMN participates in the activity of acupuncture analgesia (82). Analgesic effects as shown by an elevation of thresholds to the mechanical stimulation of the hind paw of rats were demonstrated by an elevated expression of interleukin-1-beta-mRNA in the DMN and VMN and central gray 1 hr after the i.c.v. injection of isoproterenol, a beta-adrenergic agonist. However, this may not be a unique DMN function, as other hypothalamic nuclei and even layers of the hippocampus also showed IL-1-β mRNA expression (82a).

Neuroendocrinology

Rats with DMNL (11), although retarded in both ponderal and linear growth, show normal basal levels of growth hormone (GH), insulin, triiodothyronine (T_3), corticosterone, IGF-I, plasma glucose, glycerol, free fatty acids (FFA), protein and triglycerides, and cholesterol. Circadian rhythms of GH and T_3 are normal in DMNL rats. The lesion does disrupt the plasma circadian corticosterone rhythm and results in slight changes in the circadian insulin rhythm; the latter may be feeding-related (34, 83).

Regarding the GH secretion, we repeated an earlier study that showed ultradian GH secretion was normal 39 days after lesion production (84). In our study, GH secretion was measured in DMNL rats 6 days after the hypothalamic operation (85). Not only did DMNL rats and SHAM rats show similar total GH secretion, they also exhibited no difference in their ultradian GH rhythm. This finding indicated that at a time when body weight in DMNL is just beginning to diverge from that of the controls, GH secretion is normal (in the 39-day study, GH sampling occurred after the DMNL rats already weighed less and were significantly shorter than the sham-operated rats [SHAM]).

Destruction of the DMN results in hyperprolactinemia (see Body Weight regulation), which could be due to the destruction of a DA system (11). (The effects of DMN on reproduction will be discussed in detail below). Treatments that eliminate catecholamines abolish prolactin-inhibiting factor (PIF). Also, DA released from tuberoinfundibular DA neuron terminals in the median eminence inhibits prolactin (PRL) secretion from the anterior pituitary. More recent

findings reported by Gunnett and Moore (86) have strengthened the view of a DMN role in PRL secretion. Thirty minutes of electrical stimulation of the DMN or ARC increased DOPA accumulation in the median eminence (an indicator of DA release) of ovariectomized (OVX) rats. Notably, stimulation of the VMN had no such effect. Dopamine metabolism within the tuberoinfundibular neurons also increased with DMN stimulation as evidenced by increased concentrations of DOPAC in the median eminence. Rats in which the activity of basal tuberoinfundibular DA neurons had been increased by treatment with haloperidol or decreased by bromocriptine treatment increased further following DMN stimulation as indicated by enhanced DOPA accumulation in the median eminence. It was concluded that stimulatory tuberoinfundibular efferents originate or pass through the DMN (86).

Lactation also seems to have a DMN component. Delville *et al.* (87) have reported that while lactation was correlated with a disappearance of VP receptor sites within the ventrolateral hypothalamus, it was associated with a two- to three-fold increase in the density of VP receptor binding within the DMN. The increase in VP within the DMN may be related to some undefined neurobiological aspects of lactation (87).

Evidence for the involvement of the DMN in gonadotrophin dynamics is mixed. The DMN syndrome develops in both male and female rats, which have different hypothalamic sites of gonadotropin control. Electrophysiological data from the monkey indicate that the DMN is involved in reproductive behaviors such as mounting, intromission, and ejaculation, but there is no evidence that DMN lesions in rats or other species impair male reproductive behavior (11). This is in keeping with a report that estrogen receptors (ER) and progesterone receptors (PR) were "less common" in the DMN and septum than in other hypothalamic nuclei of female puberal monkeys (88). This was found by microinjection of retrograde tracer into the median eminence and subsequent identification of ER and PR by immunostaining in separate sites of hypothalamic sections.

The DMN by definition does not belong to the classical hypophysiotropic area of the hypothalamus (89). However, even a small reduction of food caused by the DMNL during gestation may permanently affect the offspring by impairing the subsequent development of the fetus *in utero* (90). The previously demonstrated and subsequently confirmed hyperprolactinemia in DMNL rats might give rise to further changes in the neuroendocrine milieu and thus potentially affect conception, lactation, and other postnatal care. Similarly, the intrinsic hypophagia in DMNL rats might interfere with lactation on the simple grounds of their not being able to meet increasing caloric demands for nutrients.

We have, therefore, performed a study in which we investigated the effect of DMNL in both male and female rats on several parameters of reproduction and postnatal care (91). Four groups were used: 1) DMNL mothers and DMNL fathers; 2) DMNL mothers and SHAM fathers; 3) SHAM mothers and DMNL fathers; and 4) SHAM fathers and mothers. The constituent rats were bred to yield between 14–22 litters. The smallest percentage of liveborn (litter size), litter weight, mean pup weight, and percentage of pups weaned was observed in the group in which both parents were DMNL rats. These parents also cannibalized the majority of their litters. The above parameters improved when only one parent was a DMNL rat, but the measured outcomes were still significantly below that found when both parents were SHAM rats. The data also showed that the litters of DMNL mothers, but not those of only DMNL fathers, were significantly lighter than litters of SHAM rats. These differences were reduced to statistical insignificance when the mothers were SHAM and the fathers were DMNL rats.

It is well established that changes in sex hormone levels prior to, or shortly after, conception profoundly affect both sex and other fetus parameters. The data suggest that the DMNL-induced changes *in utero* were responsible for the reduced litter weight, pup weight, and subsequent survival. Although it is possible that lesion-induced postpartum alterations in behavior or milk production could also contribute to the high death rate in the offspring, DMNL rats are hyperprolactinemic, which would suggest normal or even exaggerated milk production. In support of this, those rats that survived showed excellent catch-up growth by the time of weaning. The hypothesized alterations in the mother, however, cannot explain the exaggerated death rate of the pups when both parents were DMNL rats.

At least in humans, a critical developmental period occurs during the middle part of intrauterine growth when the hypothalamic centers regulating food intake are differentiating (90). Dorner and Staudt believe that the amount of calories available at the time of differentiation of the hypothalamic regulatory centers could influence the process of neuronal differentiation and determine the future function of the centers regulating food intake and growth.

Because of logistic limitations it was not possible to maintain the litters into maturity. This was unfortunate because Jones and Friedman (92) some time ago showed that underfeeding during the first 2 weeks of gestation, but not the third week of gestation, caused female rats to produce pups that initially had normal body weight at birth and weaning but, subsequently, became hyperphagic and developed significantly heavier body weights as a result of increased adipocyte hypertrophy. If it is true that DMNL rats, as we have hypothesized, are not underfed and underweight, but merely "scaled down" in size about a new "organismic" set point (11, 93), their neurally intact male offspring would not become hyperphagic and obese, but would remain of normal body composition and weight (91).

On another aspect of neuroendocrinology, it is now well established that the PVN releases CRH to control pituitary adrenocorticotrophin secretion. However, evidence for a role of the DMN in control of CRH secretion, *via* the

PVN, has recently emerged using discrete injections of the retrograde tracer fluoro-gold (94).

The rat has for some time been used as an appropriate model for the aging human kidney (95). Aging is associated with the development of chronic nephropathy (96), glomerular sclerosis (97), and proteinuria, which is attributed largely to an increase in albumin excretion (98, 99). We have examined kidney function in rats that received DMNL at weaning, 1 and 13 months after surgery, and found that both total protein and albumin excretion rates were significantly lower than in SHAM rats (100). The fractional contribution of albumin to total urinary protein was also decreased in DMNL rats. Histological examination of the kidneys correlated with proteinuria (glomeruli, tubules, casts), the correlation coefficients being 0.90, 0.84 and 0.85, respectively. Normal glomerular and tubular structures were present in the kidneys of DMNL rats. The same findings were seen in rats 1 month after lesion production. Since total calorie restriction (101) and dietary protein restriction (102) have long been recognized to reduce the development of proteinuria and renal histopathology in rats, it is possible that the "protection" that DMNL affords this rat model is due to the reduced food intake. However, food-restriction studies usually employ a food intake that is 60% of the *ad libitum*–fed controls (103), whereas DMNL rats eat 70%–85% of what SHAM rats consume (11). Alternatively or additionally, a neurogenic mechanism might be involved somehow as well, as neurons in the DMN exhibit robust responses to electrical stimulation of afferent renal nerves (104). The stability of plasma creatinine and creatinine clearance over 13 months of life in all four groups (male and female DMNL and controls) suggests that whole kidney hyperfiltration was not present. Although 1-year-old rats may not be considered "aged," we do consider the observed changes in kidney structure and function an "anti-aging" effect of DMN lesions.

Van Liew *et al.* (100) further suggested that, in addition to the DMNL protection of rats from age-related kidney pathology, DMNL also may exert "anti-aging" effects on other parameters known to change with age. We have studied one such parameter, IGF-I (insulin-like growth-factor-I), as the IGF-I carrier, IGF-I-binding protein-3, decreases with age (105–107). The release is GH-dependent, and pulsatile GH secretion decreases with age (108). Changes in body composition that occur with aging may, therefore, be due, at least in part, to a diminished GH-IGF-I axis (109). In our study, male and female weanling rats were given DMNL and then maintained for 1 year (110). At the end of a year, the DMN rats exhibited significantly higher IGF-I levels than their respective SHAM rats. The male DMNL rats had higher IGF-I values than the female DMNL rats. Plasma concentrations of IGF-II were also significantly higher in DMNL than in SHAM rats but only in females. These data suggest that DMNL prevents the age-associated decreases in IGF-I and IGF-II. Whether this is a direct lesion effect or an indirect occurrence associated with the rat's hypophagia is not known.

One mechanism for these observed changes might be a lesion-induced imbalance between GH release and inhibitor hormones. We have shown previously that basal release of newly synthesized GH from the pituitaries of DMNL rats 20 days after lesion production (at weaning) exceeded that of SHAM rats (111). The locus specificity of this phenomenon was demonstrated by the fact that bilateral lesions in an area between the VMN and DMN did not alter newly synthesized GH release. However, the role of the DMN in GH dynamics is complicated as the ARC is thought to play a primary role in GH secretion. Furthermore, the DMN contains high amounts of DA (112). Evidently, destruction of the DMN by lesions would remove DA, which in turn would prevent an age-related decrease in pulsatile GH secretion and thus IGF-I formation as we have measured experimentally. As noted earlier, ultradian GH secretion is normal after DMNL, but pulsatile GH secretion has not yet been measured, leaving open the possibility that it may be decreased to levels seen in intact aged animals. Thus, it will be necessary to explore this possible mechanism for the lack of decline in IGF-I with age in the DMNL rat.

Ingestive Behavior

The earliest reports on a role of the DMN in feeding date back to the Hess group in Zurich (113). More systematic studies, using lesioning techniques to explore the role of the DMN in feeding, began in the early 1970s. We have since shown that DMNL produced by electolytic techniques (9), kainic acid (KA) (114), and/or ibotenic acid (IBO) (115), result in weanling or mature rats that manifest hypophagia and hypodipsia in absolute terms, but are normal when referred to body weight and metabolic mass. The DMNL rats suffer from some deficits in short-term food intake control mechanisms (see below), but long-term control mechanisms appear to be intact, as DMNL rats competently regulate 24-hr caloric intake commensurate with their reduced size. Similarly, following food deprivation, DMNL rats respond to the caloric deficit by increasing their food intake over baseline levels. We have noted that at times this food intake elevation exceeds that of SHAM rats, which may indicate that DMNL rats are responding more robustly to a severe caloric deficit than the controls are (114–115). DMNL rats respond to dietary taste consistency and dilution manipulations as do SHAM rats and are also not finicky. DMNL rats select the same percentage of macronutrients as do SHAM rats. When offered a choice, DMNL rats prefer a nutritionally complete, but unpalatable, diet over a nutritionally incomplete, but palatable diet, and thus respond like controls. However, under certain testing conditions, DMNL rats overconsume high-carbohydrate diets. The reason for this is uncertain but may be related to their attenuated ability to sense changes in systemic carbohydrate metabolism. Consistent with this possibility (116), DMNL rats have been found to exhibit short-term feeding responses to insulin in-

jection that are somewhat delayed (i.e., blood glucose levels in DMNL rats may not decline). However, long-term responses were found to be comparable to those of controls (116). Alternatively, the DMNL rat may find carbohydrates highly palatable.

DMNL rats generally show a normal efficiency of food utilization (i.e., body weight gained for amount of food eaten) which is in accordance with normal activities of pancreatic and intestinal digestive enzymes (117). Feed efficiency was further studied in DMNL rats fed *ad libitum* for either 42 or 55 days (118–119). Subsequently, these rats were food-restricted for 28 and 24 days, respectively. Upon return to the *ad libitum* feeding regimen, their body weights recovered, albeit to their normal lower growth curves, and they showed similar intermediary-metabolic parameters as did SHAM rats. Since we thought that one of the factors influencing recovery after body weight restriction could be the time between DMNL production and the start of restriction, we designed an experiment in which this time was shortened to 25 days, the restriction time being the same as before (i.e., 28 days). Notably, DMNL rats so manipulated showed an initial pronounced "overshoot" in food intake (118) which they had not done in the two previous studies (119–120). However, this response was similar to that previously found following a 1-day fast (114–115). Also, in contrast to the two previous studies, efficiency of food utilization was normal. We concluded that, although DMNL rats respond to food restriction and recovery like similarly treated SHAM rats, the duration of the *ad libitum* feeding period that preceeds restriction and/or the absolute age of the animals at that time do indeed affect some parameters. This may be related to the fact that different aspects of the DMN syndrome declare themselves in a sequential rather than a simultaneous manner (118).

Persuasive evidence (116) that the DMNL-induced hypophagia is not due to the loss of a feeding system, but rather a response to a lower body weight, has come from a study, in which, we severely (i.e., 50% of controls) restricted the food intake of a group of weanling rats for 21 days (121), while a control was fed *ad libitum.* At the end of 21 days, one subgroup of restricted rats, which were below the body weight that DMNL would normally produce in a rat of that age, and one subgroup of normal weight rats received electrolytic DMNL. Corresponding restricted and *ad libitum*–fed groups were SHAM. Following surgery all rats were fed *ad libitum.* Upon refeeding, the formerly restricted DMNL rats showed a dramatic (i.e., four-fold) increase in body weight compared to the previously *ad libitum*–fed rats with DMNL, a finding which confirmed and extended our earlier study (116). Rats that had received DMNL after food restriction also utilized food energy more efficiently than rats that had received DMNL at a "normal" body weight.

We concluded that DMNL rats are fully capable of increasing their food intake immediately after lesion production if their body weight at that time is below their "set point." Evidently, their hypophagia under *ad libitum*–normal body weight conditions is not due to interference with a feeding system, but rather may be a stratagem to decrease their body weight to a new, lower "set point" (116, 121).

Although the response of LHAL rats to an amino acid–imbalance (IMB) diet had been investigated some time ago (122–123) studies into the role of the DMN and VMN in such diets have been reported only recently. Using threonine- and isoleucine-IMB diets, Gietzen *et al.* (124) reported that the VMN showed a 22% increase in NE in the rats fed a threonine IMB diet and a 63% increase in rats fed an isoleucine-IMB diet. Both her work and that of Anand *et al.* (125), the latter based on single-unit activity in the LHA during protein hydrolysate infusions, indicate that the LHA is not critical for the depression of food intake with protein and IMB.

A recent series of studies (126), using c-*fos*–like immunoreactivity (c-FLIR) expression as an indicator of neural activity in IMB diet–fed rats, revealed cFLI in the DMN. Our group subsequently has shown that this nucleus may indeed be involved in the early (first 3 hr) detection of an IMB diet, and that an intact DMN is also necessary for the proper long-term adaptation to an IMB diet (127). Tropisetron, a 5-$HT_{3,4}$ receptor blocker, attenuates the hypophagia produced by ingesting an IMB diet. It was shown that tropisetron and DMNL produce an additive effect on the short-term intake of an IMB diet, which demonstrates that the DMNL and tropisetron act through different mechanisms (127).

The DMNL rat (9), although hypodipsic, consumes adequate amounts of water for its size, has normal percent carcass water, secretes a water load normally, and shows a normal water/food intake ratio. Thus, the DMNL rat did not show a "relative chronic dehydration" (127a) seen in rats with VMN lesions (L) (2) and LHAL rats (6). DMNL rats also show normal plasma osmolality, Na^+ and K^+ levels, and respond normally to extra- and intracellular thirst challenges and combinations of both. Also, they are not prandial drinkers or eaters and respond normally to the injection of angiotensin II (128).

An adipose tissue–derived signal proposed by Kennedy in the 1950s (128a) was thought to act as a feedback signal on the VMH. Supporting this hypothesis were findings on rats with VMNL that had been parabiosed to intact rats. These hyperphagic VMNL rats failed to respond to their own signal and became fatter. On the other hand intact parabionts did respond to a hypothesized adipose signal from their VMNL partner and reduced food intake and lost body weight (129). This elusive signal was finally identified as a product of the *ob* gene and named Leptin (130). Numerous laboratories immediately began to define leptin's mode of action and the site at which leptin acted. Initial focus centered on the mediobasal hypothalamus (131–134) and the choroid plexus (135).

Although previous studies had localized leptin recep-

tors in the choroid plexus (135) using transcriptase PCR, more recent work by Schwartz *et al.* (136) used *in situ* hybridization to investigate the regional pattern of leptin receptor gene expression. They did indeed observe leptin receptor mRNA densely concentrated in the ARC, with lower levels being present in the VMN and DMN. Quite notably, icv injections of leptin (3.5 μg) during a 40-hr fast significantly decreased levels of mRNA for NPY in the ARC (−24%) and increased levels (30%) of mRNA for CRH in the PVN. This study not only implicates the DMN (and the other hypothalamic nuclei involved in energy homeostasis) in a leptin-activated system, but also shows that the effects of leptin are two-fold: a decrease in food intake, *via* a reduced NPY expression, and an increased inhibition of feeding *via* enhanced CRH (136).

In the same year, another report confirmed the role of the DMN in leptin-induced action and in addition revealed the action of another gut peptide, glucagon-like peptide-1-(7–36) amide (GLP-1) (137). This peptide is secreted from the distal portion of the gut (138) (i.e., by the L-cells) when nutrients enter the gastrointestinal tract (139), and also by neurons in the CNS (140). Administration of GLP-1 into the third ventricle causes a reduction in food intake (141).

Intracerebroventricular administration of either leptin (90% pure in 3.5 μl) or GLP-1 (10.0 μg) 3 hr before lights off caused a significant reduction, 53% and 63%, respectively, of food intake (FI) over the first 2 hr of the dark phase. In different animals c-*Fos*-like Immunoreactivity (c-FLIR) was studied. Both leptin and GLP-1 elevated c-FLIR in the PVN and the central amygdala. Additionally, leptin selectively elevated c-FLIR in the DMN, whereas GLP-1 selectively elevated c-FLIR in the NTS, AP, lateral parabrachial nucleus, and ARC. The fact that most of the c-FLI after administration of the two peptides was observed in different areas of the CNS suggests that the two peptides have different roles in the CNS regulation of food intake and body weight. Specifically, since the levels of leptin in the plasma did not change acutely with food consumption in meals (142), leptin is an unlikely candidate for regulating the termination of ingestive bouts (i.e., short-term aspects of food intake). On the other hand, GLP-1 levels in the periphery rise in response to ingestion (143–144) and it is, therefore, conceivable that GLP-1 is involved in short-term regulation of food intake (i.e., meal terminations). Leptin may thus be a long-term body weight–regulatory factor, whereas GLP-1 acts on short-term food intake (137).

More recently, Elmquist *et al.* (145) have provided further evidence for a role of the DMN in the leptin-responsive circuitry of the hypothalamus, also by using c-FLIR. Systemic (intraperitoneal, i.p.) administration of leptin to mature male rats activated nuclear groups in the DMN and the ventrobasal hypothalamus, including the ventral premamillary nucleus and VMN. They observed c-FLIR in the dorsomedial part of the VMN, but not in the ventrolateral part. Prominent c-FLIR was also noted in the caudal DMN, but not in the caudal ARC. There was, however, activation in the parvicellular part of the ARC; the latter is known to project to autonomic preganglionic neurons.

These data are in accord with the previously noted findings by Van Dijk *et al.* (137) who also found c-FLIR leptin cells in the DMN, PVN, and central nucleus of the amygdala after icv injection of leptin. However, since circulating leptin is thought to be excluded by the blood-brain barrier, the Elmquist model is fundamentally different.

Circulating leptin may enter the brain through one of the circumventricular organs (i.e., the median eminence) and thus contact receptors directly. Such receptors reside on neurons of the overlying ARC and VMN, and perhaps even the more distant DMN and PVN, but it is considered unlikely that they reach deeper brain structures (146).

The lack of activation of the ARC, which is so close to the median eminence and has the densest concentration of leptin receptors in the brain (147), is baffling, however, this does not necessarily imply that the ARC is not involved in a leptin response. It is known that leptin administration downregulates NPY mRNA (136); these ARC neurons could be inhibited by leptin and thus would not express c-FLIR. Evidently, these and the Van Dijk data support the hypothesis that leptin may act *via* the ventrobasal hypothalamus on a number of parallel circuits that are involved in both neuroendocrine and autonomic regulation of energy homeostasis. These and Elmquist's data also suggest that the DMN is involved in one of these circuits. In a recent communication, Elmquist *et al.* (147) found that "many neurons in the hypothalamus, particularly in the dorsomedial hypothalamic nucleus (DMH) express Fos after systemic administration of leptin, and a very high percentage of these neurons project to the PVN." *Via* the PVN, whose parvicellular nuclei are a major source of autonomic preganglionic projections, leptin levels can bring to bear their influence on BAT, gastrointestinal motility, metabolic rate, and cardiovascular status.

Body Weight Regulation

Rats with electrolytic, KA, or IBO lesions in the DMN weigh less and are shorter than appropriate SHAM rats. However, they exhibit normal percentage body composition (9) and defend their lower-than-normal body weight against externally imposed excursions (114, 116, 125). Organ growth relative to metabolic size is normal except for the liver, which is reduced in weight (148). These findings suggest that DMNL rats are governed by a "true" body weight "set point" rather than a body fat set point (116). In stark contrast, rats with VMNL or LHAL show a change primarily in body fat (2, 5–6).

Based on the findings above and others (to be discussed later), it was hypothesized that the DMNL rat is governed by an "organismic" set point (2, 93, 149), which results in a "scaled-down" animal that is capable of functioning normally as shown by normal responses to nutritional, endocrine, and body weight–regulatory challenges (11).

If the DMNL rat were indeed governed by an organis-

mic set point its reduced food intake would be "normal" for its lower body weight "set point" and its readjusted—but "normal"—homeostatic capacity. Therefore, if a SHAM rat were to be given the same amount of food as that eaten spontaneously by a DMNL rat, it should show evidence of the consequences of underfeeding.

This possibility was explored by pair feeding a group of SHAM rats to DMNL rats for 12 days, while a second group of SHAM rats was presented with food *ad libitum.* Notably, whereas DMNL rats and SHAMS given food *ad libitum* showed comparable percentages of body fat and protein, the pair-fed controls exhibited reductions in both parameters (150). However, the body composition in this latter group recovered after 6 weeks of pair-feeding (151). The pair-feeding in these two studies was done in two daily feeding sessions, one in the morning and the other in late afternoon (i.e., animals were meal-fed) which does not correspond to the normal nocturnal feeding pattern of the white laboratory rat.

More recently, these experiments were repeated using a computerized feeding system. This system presented the "yoked" hungry pair-fed SHAM rat with a food pellet every time the hypophagic DMNL rat consumed a pellet. This guaranteed not only a precise quantitative, but also a synchronized temporal feeding pattern (152). In one such experiment, which lasted 11 days, the DMNL and SHAM rats showed the same carcass fat and protein percentages, plasma FFA, insulin, corticosterone, and T_3 levels. Evidently, the altered body composition of the pair-fed rats of the earlier study was a result of the manner in which they were fed (meal fed). However, plasma thyroxine (T_4) levels were significantly reduced in the pair-fed SHAMS, a typical finding of undernourished rats (152). When a second experiment was extended to 3 weeks, the pair-fed SHAMS had the same percentage carcass fat as the DMNL rats, however, they were hypoinsulinemic, another indicator of undernutrition. On the other hand, their plasma T_4 concentrations had now normalized. Plasma corticosterone levels were again comparable among the three groups. These data support the suggestion that DMNL rats, despite their reduced body weight and hypophagia, do not show the physiological changes observed in food-restricted rats. Thus, while experiencing a relative hypophagia, the food intake of the DMNL rat is commensurate with its size. This is taken to strongly support the concept of an "organismic" set point (152).

Several experimental animal models resemble the DMNL rat. One of these models is generated by the ingestion of lead (Pb) in post-weaning rats. Thus, Hammond and his group (153) have reported that weanling rats given Pb in drinking water will become hypophagic and growth-retarded, an effect that has been known from previous studies in human infants and young children. Hammond (154) noted that the Pb-induced hypophagia and reduced growth were similar to that seen after DMNL. It has been proposed that the Pb-induced growth retardation is due to either a reduced synthesis/release of GH or a depression of food intake (155). However, Hammond *et al.* (153) have shown that injections of GH and T_4, which are known to ameliorate growth retardation produced by hypophysectomy, failed to correct Pb-induced growth retardation. DMNL rats have normal GH secretion. An alternative interpretation of the Pb effect was that the metal inhibits GH, T_4, and IGF-I's normal proliferation effect on tissues (156); all three hormones are normal in DMNL rats. However, in a more recent study Hammond *et al.* (155) have eliminated this hormone attenuation possibility by obtaining a complete alleviation of Pb-induced growth repression by providing rats with additional calories by stomach tube. In addition to reduced food intake and body weight, Pb-exposed rats are also hyperprolactinemic (157), an abnormality also noted in the DMNL rat (158).

Another experimental model resembling the DMNL rat—reduced food and water intake, body weight and a defense of the lower body weight—is the rat exposed to sublethal doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Both intravenous (i.v.) and, even more so, icv administration of the substance produce this effect (159). Whereas numerous authors have attributed TCDD toxicity to an action on peripheral organs, no morphological lesions have thus far been identified in TCDD-poisoned rats. A CNS site of action was deduced from the fact that icv administration is more effective than i.v. injection. A recent report by Russell *et al.,* (160) also implicates the CNS (i.e., an increased turnover of DA in the hypothalamus). Nevertheless, a direct effect of TCDD on the appetite-regulatory area of the hypothalamus has been denied by Stahl and Rozman (161). Another resemblance of the TCDD-poisoned rat to the DMNL rat and the Pb-poisoned rat is the normalcy of the efficiency of food utilization during maintenance of the treated animals' subnormal body weight (162). Like the DMNL and Pb poisoned rat, the TCDD-treated rat also demonstrates a long-term hyperprolactinemia. In the TCDD-treated rats, the hyper-release state is preceded by 7 days of hypo-secretion of prolactin. This has been attributed to a TCDD-induced hypothalamic release of DA (the PIF) that ultimately leads to a permanent depletion of DA and subsequent hyperprolactinemia. A DMNL-induced depletion of DA was proposed some time ago (158, 160) to account for the hyperprolactinemia. It is possible, but not proven, that the hyperprolactinemia caused by TCDD may involve the DMN.

Yet another model of reduced food intake and growth retardation that resembles the DMNL rat is created by irradiating the rat's head with X-rays (163–165). Notably, bioassayable thyrotropin, gonadotropin, and somatotrophin relative to pituitary wet weight were not significantly changed in the stunted head-irradiated rat (166); shielding the pituitary from the x-ray beam also did not alter the degree of stunting (167). Although rhythmic secretory patterns of GH were normal in the stunted rats, their integrated GH secretion was reduced (167). However, treatment with

GH and/or T_4 from ages 30–40 days failed to affect body size (168). Mosier has pointed out that the area irradiated in their studies included the DMN and the substantia nigra, and that destruction of these areas by electrolytic lesions results in growth curves resembling those of the irradiated rat. It should be noted (see above) that both rhythmic and integrated GH secretion patterns are normal in DMNL rats (169, 83–85) as is *in vitro* pituitary GH synthesis (111).

Metabolism and Plasma Substrates

In short-term studies, DMNL rats do not show abnormalities in the incorporation of glucose into glycogen and lipid and exhibit normal oxidation in these tissues. Rats that had received DMNL after weaning and were ovariectomized (OVX) after reaching maturity showed diminished incorporation of glucose into lipids of diaphragm, but not of liver and fat pad, when compared to SHAM-DMNL. In fat pads, glucose incorporation was reduced in DMNL-SHAM-OVX rats in comparison with SHAM-DMNL-SHAM-OVX rats. No lesion effect was noted on glucose incorporation into glycogen in any of the three tissues studied. OVX in DMNL and SHAM-DMNL rats did not alter glucose incorporation into either glycogen or lipid in any of the tissues analyzed when compared to their respective SHAM-OVX animals. Prior to OVX, DMNL rats notably showed significant reduction in food intake, but following OVX, mean food intake of both DMNL-OVX groups did not differ from their respective controls. This finding is of particular note since OVX resulted in significant weight gains compared to non-OVX controls in both DMNL and SHAM-DMNL groups. Thus, the OVX-induced weight gain cannot be attributed to elevated food intake (170). We concluded that our data support the concept that increased weight gain following OVX can be attributed to factors other than increased food intake, that DMNL lowers the body weight "settling point" (i.e., both fat and lean body mass commensurately), and that the DMNL rat is responsive to various manipulations, in this case OVX, increasing the adipose tissue "settling point" (149). Rats with both DMNL and OVX incorporated significantly less glucose into diaphragm lipid and more glucose into diaphragm glycogen than SHAM-DMNL rats that were OVX. However, rats with DMNL and SHAM-OVX showed less glucose incorporation into diaphragm lipid than SHAM-DMNL rats that were SHAM-OVX. Also, DMNL-SHAM-OVX animals exhibited a significantly reduced glucose incorporation into fat-pad lipid compared to SHAM-DMNL-SHAM-OVX rats. All other possible comparisons were not significant.

Several key enzymes in liver, diaphragm, and epididymal fat-pad tissue were found to be normal in DMNL rats versus SHAM rats. Notably, and this is in accord with the normal body composition and plasma lipid profile (see below), our data indicate that DMNL do not disrupt lipid storage and *de novo* fatty acid synthesis in adipocytes (see 11).

Six weeks after production of DMNL, white adipose tissue showed increased basal and decreased epinephrine-stimulated lipolysis. In BAT both basal and epinephrine-stimulated lipolysis were found normal in DMNL rats. Weanling DMNL rats also show normal gluconeogenesis as evidenced by normal incorporation of alanine into tissue protein, lipid and glycogen, FFA and blood glucose (171).

Since plasma substrate concentrations of metabolites generally correlate quite well with underlying changes in tissue intermediary metabolism, we determined several key plasma substrates in DMNL rats. Notably, plasma glucose, glycerol, FFA, cholesterol, triglycerides, and total protein were in several experiments found to be normal (11).

In addition to lesion studies, a great deal of information on the role of VMN, DMN, and LHA in metabolism has come from electrical and chemical stimulation studies in these hypothalamic areas. This work originated with Shimizu (172), who in 1941 reported hyperglycemia following VMN stimulation, whereas stimulation of the LHA resulted in hypoglycemia. We established some time ago (173) that VMN stimulation for short periods resulted in hyperglycemia that persists beyond the stimulation period. The rise in circulating glucose is not accompanied by an alteration in the plasma glucose disappearance rate, indicating an increase in hepatic glucose production (i.e., glycogenolysis). Notably, plasma insulin levels did not respond to the hyperglycemia during stimulation, but rose immediately upon cessation of stimulation. This inhibition is adrenal-dependent (173). Subsequent work revealed that the stimulation-induced hyperglycemia was only slightly impaired by hypophysectomy, whereas the insulin rebound at the end of stimulation was completely blocked (174). Steffens and his group (175) extended these studies to include NE stimulation and receptor blockage and exercise-induced glucose changes as well as the measurement of FFA.

Stimulation studies specifically addressing the role of the DMN in glucose dynamics have been reported recently by Zaja *et al.* (176). The reported effects were to be expected since both Ter Horst and Luiten (24, 59) and Steffens (175) on account of both anatomical and functional studies had included the DMN in a circuitry involving VMN, LHA, and PVN, such that VMN and LHA project to the DMN and the latter to the PVN. The similarity of VMN and DMN stimulation results may also be related to their belonging to the "area sympathetica b" of Kurotsu (178) and Ban (179).

Microinjection of 12.5 nmol of NE into the DMN resulted in an increase in circulating glucose and FFA levels and caused a decrease in plasma triglyceride levels, but failed to affect circulating insulin levels within 5 min of the microinjection of NE (176). However, after 20 min, blood glucose and triglycerides returned to normal control values. Microinjection of the same amount of epinephrine into the DMN produced a similar effect over 5 min. Administration of the β-adrenergic antagonist propranolol prevented these responses. Notably, microinjection of glutamate, DA, and acetylcholine failed to cause a change in the above blood-borne parameters. These data clearly show that the DMN is

part of a β-adrenergic pathway that is involved in the short-term modulation of circulating glucose and FFA (176). As noted, the data are also in good agreement with the original proposal by Kurotsu (178) and Ban (179) that the medial hypothalamus is a sympathetic area.

Using the expression of *Fos*-like immunoreactivity (FLI) as an index of neuronal activation, Dunn-Meynell *et al.* (177) examined the anatomic substrate underlying the phenomenon of sympathetic activation that followed perfusion of the forebrain with glucose concentrations that alter neither plasma insulin nor glucose levels. Three hours after a 60-min infusion *via* the right carotid artery of glucose (4 mg/kg/min) the animals were sacrificed, and FLI was determined. As compared to equiosmolar mannitol-infused rats (as controls), 105% and 117% more neurons in the VMN and parvocellular PVN were FLI-responsive. There was also a significant activation of cells in the DMN but little FLI expression in the magnocellular PVN. Notably, the selective glucose response was bilateral. These findings support the view that a subpopulation of PVN neurons might activate the sympathetic outflow in the medulla and the cord. It is also apparent that the DMN participates in this phenomenon (177).

Although the DMN plays a role in feeding and glucose dynamics as shown by the above findings, Leibowitz and her group have reported that periods of food deprivation of 1–3 hr and the resultant decline in blood glucose produced a simultaneous decline in α_2-binding sites (as measured by binding of (^{3}H)p-amino-clonidine) in the PVN. Notably, the DMN, mPOA, and perifornical hypothalamus did not show this response. Since refeeding or administration of glucose to prevent the decline in plasma glucose also prevented the reduction in PVN α_2-receptor reduction, it was concluded that blood glucose has a direct effect on α_2-NE activity in the PVN and may affect feeding behavior. Evidently, the DMN is not involved in this phenomenon (180).

Sympathetic Nervous System, Brown Adipose Tissue, Diet-Induced Thermogenesis

It is now well accepted that the sympathetic nervous system regulates both dietary-induced thermogenesis (DIT) and nonshivering thermogenesis (NST) by its effect on BAT. Since the participation of the VMN and LHA in this phenomenon is now a certainty (181), a role for the DMN has been suspected since the early work by Niijima (182).

Following icv injection of cholecystokinin (CCK) in anesthetized rats, Yoshimatsu *et al.* (183) observed increased firing rates in sympathetic nervous system nerves to BAT. Similar responses were noted (184) after microinjection of CCK into VMN and LHA. Notably, the DMN was silent to this manipulation. It is therefore likely that the VMN and LHA, but not the DMN are involved in mediating the BAT stimulating effect of CCK injections (see Ref. (186)).

The iv administration of glucose, but not fructose, mannose, or galactose enhanced sympathetic nerve activity to BAT (182). The authors take the time lag between the peak of blood glucose and the peak discharge rate in the sympathetic nerves as an indication that it may be "related to a process in the CNS, perhaps the hypothalamus" (182); the effect might be on the glucoactive neurons located in the VMN, LHA, or DMN.

Yoshimatsu *et al.* (185) microinjected 2-deoxy-*D*-glucose (2-DG), a glucoprivic agent, into the PVN, VMN, LHA, and DMN and recorded the activity of adrenal nerves. Microinjection into the DMN and PVN produced a strong inhibition of adrenal nerve activity, whereas infusions into the VMN either induced a decrease or had no effect on adrenal nerve activity. Infusions into the ventrolateral part of the hypothalamus produced a large and long-lasting increase (greater than 60 min) in adrenal nerve activity. These findings support earlier findings reported by Oomura and Yoshimatsu (187).

The same group of authors (188) has subsequently investigated the effect of L-glutamate microinjection on BAT activity. Following the microinjection of this excitatory amino acid into the VMN, they observed a dramatic stimulatory effect in nerves supplying BAT. Microinjections into the PVN were stimulatory as well, but of smaller magnitude, whereas microinjection into the DMN resulted in a decrease of nerve activity. When microinjected into the LHA, however, two patterns were observed: An initial increase followed by a decrease or a biphasic response in nearly equal numbers of animals. Microinjection into the POA was also biphasic. These data confirm previous evidence that electrical stimulation of the VMN stimulates BAT activity (189).

We have further explored the effect of DMNL on BAT by stimulating DIT in both DMNL and SHAM rats. The animals were fed a high-fat, junk-food diet (HFJD) for 173 days and were given a 32% sucrose water solution as drinking fluid. Control DMNL and SHAM rats were fed lab chow and given tap water. Caloric intake per 100 g BW was similar, but the HFJD had higher carcass fat than their chow-fed counterparts. Notably, whereas the HFJD DMNL rats also gained carcass fat, they did so to a lesser degree than the HFJD-fed SHAM rats. Evidently, DMNL "protected" the animals from the obesifying effect of the HFJD. When considering their smaller size, DMNL had no effect on BAT size in either HFJD or chow-fed DMNL rats. Notably, BAT size was significantly increased in HFJD-fed SHAM rats. These data suggested, but did not prove, that HFJD SHAM, but not DMNL rats may be using DIT to attenuate their obesity. Alternatively, HFJD-fed DMNL rats may not be enhancing DIT, as reflected in normal BAT size, because they have not reached a degree of fatness to activate this system or the DMNL may have destroyed neurons/pathways impairing the activation of this system. Still, if this is true, the DMNL rat uses other stratagems to attenuate the obesity-producing effects of the HFJD.

The nature of these protective changes waits to be resolved. Both HFJD-fed groups, irrespective of brain status,

showed significantly reduced linear growth compared to their chow-fed counterparts. The reason for this stunting is uncertain, but may be related to their low plasma insulin levels (190).

Stress

Fear, Emotion. During the last decade it has been clearly established that the DMN and PVN are involved in escape behaviors (191, 15, 16). These two nuclei are central to a "fear circuitry" that courses from the amygdala through the hypothalamus to the mesencephalic central gray and the surrounding tegmentum (192). In an extensive study using DMN IBO lesions, Inglefield *et al.* (14) subjected male mature rats to several stress tests including the open field test, elevated plus-maze, environment-specific social interaction test, and acoustic startle test. In the open field test, the authors observed nongeneralized increases in motility parameters in the DMNL rats, with differences occurring in the latter two thirds of the test (i.e., when the situation was most novel). During the first 3 min of the test session, both DMNL and control groups had similar ambulatory scores.

Enhanced ambulation of intact rats that are inexperienced with the open field situation has also been reported following administration of the anxiolytic drug benzodiazepine (193). Anxiolytic drug administration is known to increase social interaction in the unfamiliar environment (194). In the elevated plus-maze test, DMNL rats showed a classic anxiolytic response with a greater proportion of entries into (and more time spent in) the open arms of the maze. This test has been established as a sensitive index of the level of anxiety in rats (195). Treatment with benzodiazepine increases the percentage of entries and time spent on the open arms (195). Evidently, the DMN mediates a similar anti-anxiety effect. Testing for environment-specific social interaction, unlike the maze test, did not reveal an anxiolytic effect of DMNL. In this test, the DMNL rats did not show greater interaction in the unfamiliar environment, compared to the controls.

The acoustic startle test revealed a significant enhancement of startle responsiveness in DMNL rats with the trend for the relative efficacy of pre-pulses to inhibit the startle response. An enhancement of the startle amplitude is known to occur in relation to conditioned fear and background noise (196). The authors concluded that the DMN, which along with the adjacent dorsal area is situated within several diverging functional systems, may coordinate the actions of the specific brain nuclei and thereby be involved in both behavioral and autonomic responses to a challenge. Evidently, lesions in the DMN disrupt behavioral responses to novel and challenging environments in a disinhibitory fashion (14). The role of NE, DA, and 5-HT, was assessed by Sekhar *et al.* (198) who subjected rats to the potentiating startle test and foot shock. Notably, the startle-test rats, but not those subjected to mere foot shock, exhibited significantly elevated NE and DA levels in the DMN compared to cage controls, which suggested decreased DMN transmitter release. On the other hand, foot shock significantly elevated 5-HT levels in the DMN. Evidently, NE and DA in the DMN are involved in the motion-related and fear-associated role of the nucleus.

The same group of authors (197) demonstrated that connections between the OVLT and DMN may be involved in lactate panic disorder. Patients with panic disorder experience panic attacks after iv lactate infusions by an as yet unexplained mechanism. Implantation into the DMN of a GAB synthesis inhibitor, L-allolyglycine, and another chronic injection cannulae into the OVLT was used as a rat test system. A direct injection of lactate into the OVLT elicited a robust anxiety-like response in the rats whereas an injection of tetrodotoxin into the OVLT completely blocked the lactate-induced response. It is likely that the OVLT may be the primary site that detects lactate infusions and activates an anxiety-like response in a compromised DMN.

The same group of authors (199) recently reported findings of a more detailed follow-up of the above experiment. Only rats subjected to the full fear-potentiating startle test—and not the other tests of anxiety (foot shocks or acoustic startle)—showed a significant increase in NE and DA in the DMN 24 hr after the test. A time-course study revealed that such elevations were present 12 hr after the test. Notably, tyrosine hydroxylase activity in the DMN of fear-potentiated startle rats remained in the normal range. An *in vitro* release study found a significant decrease in potassium-stimulated release of NE from the DMN and, in conjunction with the above enzyme test, allowed the conclusion that the change in DMN catecholamine concentrations upon fear may be the result of a decrease in NE and DA release and not an increase in synthesis of the catecholamines (199). These findings suggest that there is some factor/event inhibiting the release of the two catecholamines from the DMN.

Electrical stimulation of the DMN has also been used as a tool to impose emotional stress. In the initial phase of a 120-day study using rabbits, Nikolaev *et al.* (200) recently reported an (expected) rise in ACTH, cortisol, catecholamines and elevated levels of lipid peroxidation and antioxidative enzymes. With time (60–120 days), the former three hormones decreased, whereas the concentration of parathyroid hormone and angiotensin I levels rose. A significant positive correlation was found between the blood levels of the hormones and the rate of lipid peroxidation in plasma and myocardium. Concomitantly, the levels of antioxidative enzymes decreased progressively, and all rabbits showed myocardial cell necrotic foci (200a). It is concluded that "disturbances in the regulatory mechanisms of hypothalamic mediator systems lead to an increase in excitability and to transformation of the adaptive pattern of hormonal changes into pathological mechanisms of prolonged emotional stress" (200).

Physical contact in nature between prey and predator elicits a response that is called tonic immobility (TI). In the

laboratory this response can be initiated by postural inversion and brief postural contention of its movements. An involvement of the DMN in this phenomenon was demonstrated by showing that a microinjection of carbachol into the VMN and DMN promoted a reduced duration of 11 TI episodes. Pretreatment of guinea pigs with atropine showed that this action of carbachol is mediated by muscarinic receptors in the anterior and hypothalamus and VMN but not in the DMN (201).

Cardiovascular Aspects. In addition to the evidence involving the DMN in circulatory control mechanisms (detailed in our 1987 article [11]) numerous new reports on this aspect of DMN function have recently been published. For example, Nagao *et al.* (201) clarified the effects of destruction of the DMN and midbrain and stimulation of the medulla oblongata on intracranial pressure in experimentally induced subarachnoid hemorrhage. Using cats, the authors produced acute subarachnoid hemorrhage by injection of autogenous blood (3–7 ml) into the cisterna magna. In one group of cats, bilateral lesions were produced in the DMN and the reticular formation of the midbrain 1 hr after production of the subarachnoid hemorrhage. In a second group of cats, in addition to the above procedures, the reticular formation of the medulla oblongata was electrically stimulated bilaterally.

The majority of animals in both groups showed transient small increases of intracranial pressure and local cerebral blood volume following coagulation of the DMN or the midbrain reticular formation or during stimulation of the reticular formation of the medulla oblongata. In the animals whose intracranial pressure remained approximately 20 mm Hg or below, the progressive increase in intracranial pressure was never evoked. In several animals of the first group, however, whose intracranial pressure remained higher than 20 mm Hg, sequential destruction of the DMN and the midbrain reticular formation induced a stepwise increase in intracranial pressure to 40–80 mm Hg and consequently resulted in acute brain swelling. In only one animal of the second group, the intracranial pressure fluctuated about 20–40 mm Hg, and a biphasic increase in intracranial pressure to 100 mm Hg was repeatedly evoked by stimulation of the reticular formation of the medulla oblongata. The authors concluded that in the condition of increased intracranial pressure produced by subarachnoid hemorrhage, lesions in the DMN and reticular formation of the midbrain and stimulating condition of the midbrain reticular formation may result in acute brain swelling (201). This may be interpreted as indicating that medullary stimulation increases blood pressure by separate pathways from the DMN.

Recent reports also implicate the DMN in a very specific aspect of circulatory control (i.e., myocardial flood flow (202)). Using urethane-chloralose anesthetized rabbits that were vagotomized and immobilized by galanin triethiodide and artificially ventilated, the authors injected radioactive microspheres into the left atrium and unilaterally stimulated the left or right DMN electrically. Notably, they observed an asymmetrical effect, the right DMN stimulation resulted in no significant change of left ventricular blood flow, whereas the left DMN stimulation produced a significant flow reduction. No significant change was seen in the right ventricular blood flow. Both left and right DMN stimulation resulted in a rise in mean arterial blood pressure and a change in the epicardial electrocardiogram (EKG) ST segment, but there was no change in heart rate.

The authors also found that, in intact rabbits, DMN stimulation caused a decrease (indicating a neuronal release) in immunoreactive material of substance P (SP) in bilateral intermediolateral cell columns at the T2-T5 levels. This was accompanied by the aforementioned elevation in mean arterial blood pressure and changes in the EKG ST segment. These combined sets of data allow the conclusion that cardiovascular changes produced by DMN stimulation are mediated by the release of SP in the intermediolateral column cells of the spinal cord, an area with which the DMN has known connections (202).

Subsequently, the same group of authors (203), using the same model, investigated the effect of somatic input on the cardiac ischemia produced by DMN stimulation. Electrical stimulation of the median nerve or deep peroneal nerve partially or completely inhibited the ischemic EKG segment ST deflection that is caused by DMN stimulation (202). The inhibitory effect of median nerve stimulation was more pronounced than that of peroneal nerve stimulation. Notably, the inhibition of the ST segment changes could also be produced by intrathecal morphine injection (40 μg). When naloxone (20 μg) was injected intrathecally, the inhibitory effect of the median nerve stimulation on the DMN-stimulation-induced, ischemic-ST-segment changes was abolished. It was concluded that stimulation of the median nerve and the deep peroneal nerve can inhibit cardiac ischemia induced by DMN stimulation and that the effects of median nerve stimulation are more potent. An increase in immunoreactive L-enkephalin material in the bilateral intermediolateral cell column may mediate the inhibitory effect. Median nerve stimulation effects may also be mediated by an increase of ipsilateral L-enkephalin in the thoracic intermediolateral cell column through a segmental mechanism to inhibit sympathetic activity (203).

More recently, the same group of investigators (205) further studied the DMN-induced cardiac ischemic response and its reversal by deep nerve stimulation. They explored for central sites that might participate in this response. They reported that lesions of the nucleus accumbens (ACC) abolished the inhibiting effect of deep peroneal nerve stimulation on the pressor response and EKG segment changes induced by DMN electrical stimulation. Conversely, electrical stimulation of the ACC caused a prominent depressor effect that could be effectively lessened by microinjection of naloxone into the ventral PAG, which again supported the aforementioned opioid involvement in this response. On the other hand, electrical stimulation of the PAG is known to cause hypertension in the rat (204). Fan and Zhang (205)

lesioned the ARC of the hypothalamus and found that the depressor response following ACC stimulation almost disappeared which indicated a central, as well as peripheral, role of opioids and this response.

Although the PVN and the DMN are connected by fibers identified in the 1980s (24, 59), they do not show concordant responses to certain manipulations. Thus, microinjection into the PAG of 0.68–6.8 nmol/rat of N-methyl-d-aspartic acid (NMDA) produced a significant rise in blood pressure. When the animals were pretreated with the NMDA antagonist dl-2-amino-5-phosphonovaleric acid [0.05–5 nmol/rat] into the PVN, PAG administration of NMDA elicited a decrease, not an increase in blood pressure. Notably, injection of 2-dl-2-amino-5-phosphonovaleric acid into the DMN did not modify the increase in blood pressure when NMDA was injected into the PAG. These data suggest not only the existence of a glutaminergic connection between the PAG and the PVN regarding cardiovascular effects, but also reveals that the DMN is not involved in this phenomenon (206).

Nevertheless, the PAG has a role in the DMN's efferent control of cardiovascular function as shown recently by Van der Plaas *et al.* (207). They reported that electrical stimulation of the DMN and the ZI resulted in a significant depressor response. Furthermore, ipsi- and contralateral DMN stimulation, and to a lesser degree, ZI stimulation resulted in an inhibition of most PAG neurons. In most PAG neurons, the inhibition strictly followed the time course of hypothalamic stimulation. However, the authors also noted that non-PAG neurons participate in the hypothalamic control by the DMN of cardiovascular function (207).

Whereas electrical stimulation did not alter heart rate, DMN microinjection of the GABA, antagonist BMI (20 pmol) in urethane-anesthetized rats elicited tachycardia (208). This effect is thought to be mediated by pathways involving GABA-ergic interneurons at the level of the DMN. Inhibition of the DMN, in turn, inhibits descending vagal outflow to the heart at the level of the NTS. Notably, intra-NTS microinjection of BMI (10 pmol) or the GABA-B receptor antagonist 2-OH-saclofen reversed the tachycardia brought about by DMN GABA antagonism. The tachycardia was also inhibited by the intra-NTS injection of the NMDA receptor channel blocker MK-801 (3 pmol) or the non-NMDA antagonist CNQX (400 pmol). These data indicate that the GABA-ergic control of the heart at the level of the DMN and NTS is mediated by GABA A, GABA B, and NMDA receptors and that descending input from the DMN to the NTS involves the release of GABA (208).

Cardiovascular and neuroendocrine effects of the DMN and PVN were recently studied concurrently (18). Mature, conscious control rats subjected to air stress, showed an increase in plasma ACTH, heart rate, and arterial blood pressure. When muscimol, a GABA A receptor agonist, was microinjected into either the PVN or DMN prior to air stress it reduced the associated increases in plasma ACTH. However, only muscimol injection into the DMN attenuated the accompanying tachycardia and increase in arterial blood pressure. These data support the above-mentioned findings and show that, evidently, GABA-ergic neurons in the DMN, but not the PVN, play a role in both cardiovascular and neuroendocrine responses to stress (18).

Greenwood and DiMicco (209) also used BMI to test the hypothesis that the DMN might affect intestinal motility. Motility was measured manometrically with saline-filled cannulae in anesthetized mature rats. Indeed, reproducible and dose-related increases in jejunal and colonic motility, as well as heart rate and arterial blood pressure, were elicitable after microinjection of 15–30 pmol/15 nl of BMI into the DMN. The locus specificity of this response is secured by the fact that microinjection of BMI at sites anterior to the DMN and closer to the PVN elicited greatly attenuated cardiovascular effects, and intestinal parameters were either not changed or significantly reduced. Both vagotomy or treatment with atropine methyl bromide (1 mg/kg iv) blocked the increase in jejunal motility and reduced, but did not abolish, the colonic stimulation. BMI DMN stimulated the increase of heart rate and blood pressure were essentially unaffected by either manipulation. The data suggest that jejunal motility is increased by disinhibition of DMN neurons. The final common pathway of the DMN action is vagal *via* cholinergic fibers. Their data further indicated that colonic motility is enhanced by both vagal and nonvagal cholinergic pathways.

The authors (Greenwood and DiMicco, [209]) speculated that their findings in rats may parallel clinical observations in patients with functional bowel disorders, where it is believed that symptoms are made worse by emotional stress. The hypothalamus is thought to integrate physiological response to emotional states. Indeed, electrical stimulation of the posterior hypothalamus in humans increases heart rate and induces "a strong emotional reaction of a fearful quality" (210). It is hypothesized that activation of the DMN may be involved, at least in part, in the intestinal dysmobility that is associated with functional bowel disorder (209).

In order to resolve apparent contradictions between findings by others (210–212) on the effect of stimulating the hypothalamic "defense area" using excitatory amino acids (EAA), DiMicco and his group (15–17) tested kainate, N-methyl-D-aspartate (NMDA), and α-amino-3-hydroxy-5-methyl-5-isoxasole propionic acid (AMPA), EAA known to act more or less selectively at particular subtypes of ionotropic glutamate receptors. The authors chose to use a wide range of doses. In contrast to findings by others, they found that following microinjection into the DMN at low (0.1 pmol/50 nl to 10 pmol/50 nl) (i.e., doses at injected concentrations of as low as 2 μmol/l) these EAA caused marked increases in heart rate and modest increases in arterial pressure (similar to responses evoked by microinjection of BMI in both anesthetized and conscious rats). The effects of NMDA and kainate were shown to be mediated through NMDA and non-NMDA subtypes of ionotropic

EAA receptors, respectively. They also found that Kynurenate, a nonselective antagonist of ionotropic EAA receptors, markedly suppressed air stress–induced cardiovascular changes when microinjected into the DMN but not into sites injected lateral or posterior to the region (17). Evidently, not only were neurons in the DMN exquisitely sensitive to EAA-mediated stimulation, but the synaptic activity at local EAA receptors seemed to play a key role in generating stress-induced cardiovascular changes.

In summary, the current picture of a DMN role in cardiovascular regulation is that the DMN contains an inhibitory center that is mediated by GABA-ergic signals. Disinhibition of the DMN system by microinjection of GABA A receptor antagonists evokes responses resembling those of physiological changes of acute experimental stress (213). The opposite manipulation (i.e., microinjection of muscimol, a potent agonist at inhibitory GABAA receptors) virtually abolished stress-induced DMN-mediated tachycardia and elevation of arterial blood pressure. This system also includes excitatory amino acids, since blockade of these receptors also abolishes stress-induced DMN-mediated cardiovascular changes. Microinjection of KA, NMDA, and B-amino-3-hydroxy-5-methyl-5-isoxazol-propionic acid into the DMN elicited cardiovascular effects resembling those seen in rats under stress. Although microinjection of these drugs into the PVN also produced some effects, they were small. Evidently, there is compelling evidence for the existence "... in the immediate vicinity of the DMN ..." (213) of a set of neurons that integrate the acute central neural response to environmental stress, particularly autonomically related cardiovascular changes. The activity of these neurons is to a great extent determined by the balance of tone at inhibitory GABA A receptors and excitatory amino acid receptors (213).

Circumventricular Organs

Regarding circumventricular organs, it should be recalled that the DMN entertains direct connections with the AP. Shapiro *et al.* (214) and Hosoya and Matsushita (215) have identified the cells of origin of this projection by the retrograde horseradish peroxidase method at the rostral plane of the DMN in the dorsal hypothalamus. Visceral abdominal sensory systems play an important role in feeding behavior and energy balance (216), and the AP is a site of input of abdominal sensory systems (217). It is possible that neurons of the vagus and the NTS as well as A 2 catecholamine neurons and their central output are brought under the direct regulatory influence of the hypothalamus, specifically the DMN (215).

In this context it is significant that AP lesions and those destroying the DMN are similar but not identical. Both lesions result in body weight and food intake reduction postoperatively, but whereas the body weight of the AP-lesioned rats remains at a level of about 70%–80% of that of controls, food intake normalizes by about 60–100 days postoperatively (218). This suggests a disturbance of energy balance. The latter authors have postulated the resetting of a body weight "set point" (which is really a change in body fat set point [see above]) as the AP-lesioned animals show a significant reduction in percentage of carcass fat; DMNL rats do not show an altered percentage of carcass fat. Thus, we are dealing in this case, as with the LHA-lesioned rat, with a lowered body *fat* "set point," whereas DMNL rats have a lowered body *weight* set point (116).

In addition to projections to the AP, the DMN also provides a rich supply of projections to the OVLT and the SFO (219, 59), two other circumventricular organs. Ter Horst and Luiten (24) identified efferent connections from the OVLT to the DMN. As the circumventricular organs lack a blood brain barrier, they have been considered as mediators of peptide hormone (e.g., insulin) effects on the brain (220). The DMN may modulate hormonal feedback mechanisms *via* this mechanism (59). The majority of SFO fiber projections end in the PVN and the SON, mPOA and parastrial nuclei, as well as the DMN. It is known that each of these SFO recipient nuclei projects to the DMN (221). The DMN projects densely to these areas but sparsely to the SFO itself. "Therefore, it is difficult to avoid the conclusion that the DMN influences the output of the subfornical organ and thus influences body fluid homeostasis" (20).

The foregoing findings clearly indicate that since its first review 22 years ago, the dorsomedial nucleus of the hypothalamus has moved from a rather obscure position into the foreground of structures involved in neuroendocrine and neuroautonomic homeostasis. Certainly, in view of its role in the receptor circuitry of the newly discovered body fat regulatory hormone leptin, it will be the object of further intensive investigations by students of body weight and fat regulation as well as autonomic-neuroendocrine control mechanisms.

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