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Deficits In Water Intake In Rats With Electrolytic Lesions Of The Zona Incerta

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DEFICITS IN WATER INTAKE IN RATS WITH ELECTROLYTIC LESIONS OF THE ZONA INCERTA

by

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ABSTRACT

Previous studies have implicated the zona incerta (ZI), a diencephalic extension of the midbrain reticular formation, in the control of drinking behavior in the rat. The purpose of the present experiments was to investigate the nature of the deficit in water intake after ablation of this region. Male Wistar rats were used in the study, in 9 series of 20-30 rats each. Bilateral electrolytic lesions were made in the region of the ZI; rats were assigned to experimental or control groups for data analysis solely on the basis of histological determination of site and extent of these lesions.

Lesions of the ZI, but not sham lesions or lesions dorsal to the ZI, reduced daily water intake of rats by 20-30% with no concomitant changes in food intake or body weight. The hypodipsia was permanent, and was not caused by alterations in water requirements or excretory mechanisms. Compensatory reductions in water losses maintained fluid balance.

The reduced water intake could not be attributed to failure to respond to signals of water deficit; lesioned rats drank as much as controls to intracellular and extracellular dehydration induced by injections of hypertonic saline and hyperoncotic colloid. However, unlike control

rats, rats with lesions of the ZI appeared to restrict their daily intake to these responses to water deficit. Lesions of the ZI attenuated the ingestion of extra water observed in rats maintained on a liquid diet adequate to meet fluid requirements, and daily water intake of lesioned but not control rats closely followed changes in daily fluid need when diet protein levels were varied. It was concluded that lesions of the ZI reduced ad libitum water intake towards minimal requirements for fluid balance by attenuating secondary drinking (drinking independent of water needs for fluid homeostasis).

Since previous studies have implicated secondary factors in the control of daily patterns of ingestion, food jars and water spouts were equipped with devices to continuously record feeding and drinking activity. Lesions of the ZI, made through implanted electrodes in conscious rats, caused only a transient (4-6 hr) disruption of normal patterns of intake. Subsequently, lesioned rats ingested smaller but not fewer drinks. Furthermore, they lapped as frequently at the water spout as controls but obtained 15-20% less water per lap, suggesting an impairment in the ability to ingest fluid.

Impairment in the ingestion of water, making drinking more difficult, would attenuate secondary water intake but should also affect drinking to water deficits.

Lesioned rats drank less than controls after water deprivation when the drinking solution was made bitter by adding quinine or sweetened with saccharin, and the intake of lesioned rats was more severely attenuated by lower concentrations of quinine. In contrast to the earlier findings, these results demonstrated a subtle but significant deficit in the drinking response to dehydration as well as secondary water intake.

Further investigation confirmed that lesioned rats were impaired in their ability to ingest fluids. Lesioned animals obtained 30% less water per lap than controls when drinking after water deprivation and 55% less liquid diet per lap after water or food deprivation. Thus, it was proposed that the reduction in daily water intake and attenuation of secondary drinking after lesions of the ZI were caused by an impairment in oropharyngeal function. These results are discussed in terms of the anatomical connections of the ZI and possible deficits in sensorimotor integration. The implications of these findings for the investigation of neural substrates underlying motivated behavior are also considered.

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CHAPTER 1. INTRODUCTION

The early studies of Richter (1942) demonstrated the importance of the contribution of behavior to the preservation of homeostasis. This is particularly obvious for ingestive behaviors since a continual regulation of water and energy exchanges with the external environment is vital to life. How these behaviors are initiated and coordinated with physiological regulation is one of the most intriguing problems in the investigation of brain function. Early ablation and stimulation experiments implicated the hypothalamus as the integrating "center" of the brain for the regulation of food and water intake, but more recent evidence has demonstrated that other brain structures also contribute to these functions. The zona incerta, a rostral extension of the midbrain reticular system, has recently been implicated in the control of drinking behavior; this thesis presents the results of experiments to determine the way in which the zona incerta participates in the control of water intake and the regulation of body fluid balance.

The subject of water intake regulation and studies contributing to the present understanding of the neural mechanisms involved are reviewed in the next sections. Since this is a large and rapidly expanding field of research, a comprehensive review is impossible and only an

overview has been attempted. Wherever possible, reference has been made to some of the excellent reviews available covering particular aspects of this area. A review of anatomical and physiological studies of the zona incerta follows and the Introduction is concluded with a general statement of the problem under investigation.

1.1 Water Intake Regulation and Body Fluid Homeostasis

Water, the major component of the *milieu interieur*, is distributed throughout the body in two compartments: the intracellular fluid (ICF) located within the cells, and the extracellular fluid (ECF) including water within the cardiovascular system and the interstitial space. The volume and concentration of the fluids within these compartments must be precisely regulated despite a continual exchange of water with the environment, including mandatory water losses for solute excretion and temperature regulation. Normally body fluid homeostasis is maintained through the synergistic activity of water intake and water loss mechanisms. Although renal regulatory mechanisms are useful in reducing water excesses and contribute to the conservation of body fluids when water supply is restricted, only the ingestion of water will correct actual fluid deficits. Results obtained over the last fifty years suggest that drinking behavior may be as precisely regulated as urinary water losses.

Cellular Dehydration

The work of Mayer and Wettendorf at the turn of the century (cited by Fitzsimons, 1972) suggested that tonicity of the body fluids was an important determinant for the regulation of water intake. Mayer reported an increase in blood osmolality of water-deprived dogs and noted that allowing the dogs to drink reduced both the osmolality and the thirst. Wettendorf observed the same phenomena but, noting that the osmolality changes were small, suggested that fluids moved from the tissues into the ECF compartment and that it was the cell water loss that was the stimulus to drink. This was confirmed when Gilman (1937) demonstrated that intravenous injections of NaCl solutions elicited twice as much drinking as did injections of urea, although both increased serum osmolality. Since urea passes relatively freely across cell membranes, an effective osmotic gradient would not be created and little fluid would be drawn from the cells. These findings were further extended by Holmes and Gregersen (1950), injecting both electrolytes and non-electrolytes to confirm that the induction of thirst depended on cellular dehydration per se.

Adolph et al. (1954) reported that the rate of drinking after injections of hypertonic saline solutions was related to the rate of solute absorption, but attempts to quantify the relationship between cellular dehydration and water intake were hindered by the great variability

observed in the drinking response (Holmes and Gregersen, 1950; Kanter, 1953). The volume of water consumed was not enough to dilute body fluids to isotonicity, presumably due to the accompanying contribution of renal mechanisms. Fitzsimons (1961a) resolved this problem by demonstrating that the volume of water consumed by nephrectomized rats was precisely that needed to attain isotonicity. There was no adaptation to the stimulus since withholding water for 24 hours after injection did not reduce the drinking response, and drinking ceased in nephrectomized rats only when body fluids had been restored to isotonicity.

The similarity between the osmotic control of urinary water losses (Verney, 1947) and water intake is obvious and under normal conditions both mechanisms will act simultaneously to maintain cellular fluid volumes. The thresholds for activation of the two mechanisms are similar: a 1-2% decrease in ICF elicits drinking in dog and man (Wolf, 1950) and in intact and nephrectomized rats (Fitzsimons, 1963) and is similar to that observed by Verney (1947) for eliciting antidiuresis.

Extracellular Dehydration

It was obvious from early studies that intracellular dehydration could not account for all cases of thirst. For example, increased water intake was observed to accompany Na^+ depletion in dogs (Cizek et al., 1951; Holmes and Cizek, 1951) and man (McCance, 1936). Reduced Na^+ concentration

results in loss of fluid from the extracellular space into the urine but also into the tissues, such that the cells actually become overhydrated (Darrow and Yannet, 1935). Thus attention was directed to the role of reductions of ECF volume in the control of water intake. As the *milieu* through which all the exchanges between cells and the external environment must take place, regulation of the ECF compartment is necessary. Of particular importance is that fluid which is located within the vasculature, and elaborate mechanisms exist for preserving the pressure and volume of the circulating blood (Folkow et al., 1965; Gauer et al., 1970). Early clinical observations associated thirst with hemorrhage although the results were often equivocal (see Fitzsimons, 1971).

Experimentally a relative reduction of ECF volume can be produced by injecting a hyperoncotic colloid (e.g., polyethylene glycol) dissolved in isotonic saline into the peritoneal cavity (Fitzsimons, 1961b). This causes a sequestration of ECF in the peritoneum by a Starling mechanism without altering body fluid osmolality. Fitzsimons (1961b) reported that rats treated this way began to drink within an hour after injection. Subcutaneous injections of polyethylene glycol have also proved effective and it has been demonstrated that intravascular hypovolemia and drinking occur in proportion to the concentration of colloid administered (Stricker, 1966; 1968). A

reduction of ECF, unlike ICF dehydration, normally requires the replacement of solute as well as water and increased solute intake and preference for saline solutions have also been reported after these manipulations (Stricker, 1973).

It is likely that the receptors involved in the regulation of ECF volume are located within the vasculature, primarily in the low-pressure capacitance vessels. Stretch receptors within the distensible veins near the heart and atria have been implicated in the control of vasopressin and aldosterone secretion (Gauer et al., 1970; Fitzsimons, 1972) and ligation of the abdominal inferior vena cava in the rat, reducing venous return to the heart, induces drinking (Fitzsimons, 1964). The exact mechanisms involved, however, remain unknown.

The renin-angiotensin system (see Peart, 1969) has also been implicated in the drinking response to reductions of ECF volume. Reduced blood flow to the kidneys elicits drinking in rats, ligation of the vena cava is a less effective stimulus for thirst after nephrectomy, and the injection of renal extracts or renin elicit water intake (Fitzsimons, 1967; 1969). It appears that the effect of renin on water intake is mediated through its enzymatic production of angiotensin II. The application of small quantities of angiotensin to the brain has been shown to produce copious drinking in several species (goat: Andersson and Westbye, 1970; rat: Epstein et al., 1970; monkey:

Setler, 1971). The literature in this area is far too extensive to be reviewed here and the reader is directed to recent reviews (Fitzsimons, 1972; 1975).

The Double Depletion Hypothesis of Thirst

To summarize the preceding sections, water intake is an important component of those physiological mechanisms involved in maintaining body fluid homeostasis and drinking occurs as a regulatory response to reductions in either intracellular or extracellular fluid compartments. This has been referred to as the double depletion hypothesis of thirst (Epstein, 1973). In the laboratory various experimental techniques are available for separately eliciting either of these stimuli, but under natural conditions water loss normally involves depletion of both fluid compartments simultaneously. It has been demonstrated that the signals from the two compartments are additive in their effect on water intake (Blass and Fitzsimons, 1970). Other interactions may also occur such as the inhibition of water intake to ECF reduction before intravascular restoration is complete due to cellular overhydration (Stricker, 1969).

Not all drinking, however, can be explained by the on-off response of intracellular and extracellular regulatory mechanisms or their interactions, and Adolph and co-workers (1954) emphasized the importance of considering that drinking behavior was under the control of multiple factors. Numerous variables may modulate regulatory

drinking responses. Furthermore, there has been increasing recognition that drinking may occur in the absence of existing water requirements for fluid balance and that non-deficit factors may be important in the normal day-to-day regulation of water intake.

Modulatory Influences

Gustatory (Epstein, 1967), thermal (Kapatos and Gold, 1972), and proprioceptive (Corbit and Luschei, 1969) cues modulate the volume or pattern of drinking to body water deficits. Furthermore, under normal environmental conditions the problem of behavioral priorities is important and an animal may be subjected to various homeostatic demands in addition to the maintenance of body fluid balance. Under certain conditions it may be necessary to compromise the latter in order to meet more pressing physiological needs (Blass, 1973).

There is evidence that positive feedback may be involved in maintaining drinking once it starts (Oatley, 1973) and Kissileff (1973) has suggested that rats may normally ingest some fixed minimum volume of water (0.5-1.0 ml) which can be adjusted upward in the presence of greater water requirements. Also relevant to the present discussion is the phenomenon of preabsorptive satiety. Final satiety obviously is achieved by the complete restoration of body fluid balance, but in many species drinking may cease before any significant absorption

of ingested fluid has taken place (Fitzsimons, 1972). Some form of oral metering may be involved (Emmers, 1973) which would enable an animal to complete the act of drinking quickly and avoid the overhydration that would occur if drinking continued until adequate absorption was detected. Thus various modulatory influences related to internal and external cues as well as neural programming may participate in the control of drinking to water deficit signals.

Secondary Drinking and Ad Libitum Water Intake Regulation

Drinking does not occur only under conditions of body fluid deficit. Fitzsimons (1972) has divided the situations in which drinking occurs into two main categories: primary drinking, when there is a relative or absolute deficit in one of the body fluid compartments as discussed above; and secondary drinking, in which there is no apparent internal need for water for maintaining fluid homeostasis. Numerous stimuli such as taste (Ernits and Corbit, 1973), arousal (Fitzsimons, 1972), or schedules of reinforcement (Falk, 1969) may initiate drinking in the absence of dehydration. Animals will learn to drink to relieve discomfort such as following injections of lithium chloride (Smith et al., 1971) or procaine (Mineka and Seligman, 1975), or to facilitate some other behavior. For example, following surgical (Kissileff, 1969b) or pharmacological (Chapman and Epstein, 1970) desalivation, eating dry food becomes

difficult, but rats will learn to alternate bites of food with small drafts of water to moisten their mouths.

There is also evidence that animals can learn to associate environmental cues with dehydration; drinking in response to control injections has been observed after repeated testing with hypertonic saline injections, and this association appears particularly resistant to extinction (Mineka and Seligman, 1975). A similar learning process or innate mechanism may be involved in the drinking response of rats to increases in ambient temperature. Although such increases eventually lead to increased water losses (Hainsworth et al., 1968), there is evidence that the drinking occurs prior to these losses and may be initiated by the heat stimulus per se (Grace and Stevenson, 1971).

Although in the past the greatest emphasis has been placed on primary water intake, there has been increasing recognition of the importance of secondary drinking in the day-to-day maintenance of body fluid balance. There is evidence that when water is freely available, animals may normally ingest more than is actually required to maintain water balance, since rats restricted to 65% of their usual daily water intake continue to eat and gain weight normally (Dicker and Nunn, 1957). The consequences of excess intake in an animal with functional excretory mechanisms would be minimal. To determine the extent to which dehydration governed ad libitum water intake, Fitzsimons (1957; 1971)

implanted chronic gastric catheters in rats. When volumes of water equal to their normal daily intake were continuously infused into the stomach, drinking was reduced by only one-third. The nocturnal predominance of the drinking persisted, even when the amount infused at night was doubled, indicating that the need for water does not adequately account for the pattern of intake or volume ingested.

When food and water are available ad libitum, there is a close temporal relationship between feeding and drinking in the rat, since 70-75% of the total daily water intake occurs within ten minutes before or after a meal (Kissileff, 1969a). Secondary factors may be important determinants of this relationship. Primary deficit signals will arise following the ingestion of food due to the influx of solutes (Novin, 1962) and secretion of digestive juices (Lepkovsky et al., 1957), but there is evidence that drinking may precede the fluid shifts (Oatley and Toates, 1969; Oatley, 1973). Furthermore, a rat may consume up to 25% of its meal-associated drinking before eating begins (Fitzsimons and LeMagnen, 1969; Kissileff, 1969a).

Fitzsimons and LeMagnen (1969) have proposed that under normal stable conditions rats may learn to associate the ingestion of food with their subsequent needs for water. When daily fluid requirements were increased by increasing diet protein content, there was an immediate

increase in water consumption but the percent of drinking occurring in close temporal association with meals fell. By the third day, however, the close relationship was re-established. When fluid requirements were reduced by withdrawing the excess diet protein, drinking decreased only slowly and less completely than expected, but the relationship between eating and drinking was maintained throughout. Fitzsimons and LeMagnen concluded that learned associations between feeding and drinking can be established and at least partially determine total fluid intake. When water requirements were reduced, these learned associations persisted.

Thus, as proposed by Adolph et al. (1954), there is much evidence that multiple factors participate in the control of water intake. The greatest emphasis in the past has been on drinking in response to body fluid deficits and intracellular and extracellular controls have been described. More recently, however, there has been greater recognition of the importance of secondary controls operating independently of existing deficits for water and perhaps even anticipating them.

1.2 Neural Control of Water Intake

Role of the Hypothalamus

Excellent reviews of the early studies implicating the brain in the control of water intake have been provided by Stevenson (1969) and Fitzsimons (1973). Nothnagel (cited by Fitzsimons, 1973) postulated the existence of a "thirst" center as early as 1881. He described the case of a thirty-five-year-old man who experienced acute thirst and engaged in excessive drinking after a head injury, and suggested that this was due to damage to some critical hindbrain site. With the early clinical interest in diabetes insipidus and the subsequent demonstration of the role of the basal diencephalon in this phenomenon, attention was directed towards the hypothalamus as being an important area for the preservation of water balance. The application of the stereotaxic technique to the exploration of the hypothalamus demonstrated its role in ingestive behaviors; Hetherington and Ranson (1942) reported hyperphagia and obesity after electrolytic lesions of the medial hypothalamus and Anand and Brobeck (1951) reported aphagia after lesions of the lateral hypothalamus (LH). Teitelbaum and Stellar (1954) first reported that adipsia accompanied the aphagia after LH ablation. Further study demonstrated that the adipsia was not merely secondary to the aphagia (Morrison and Mayer, 1957) and that with small lesions the adipsia could be

obtained in the absence of aphagia (Montemurro and Stevenson, 1957).

The effect of LH lesions on ingestive behavior in rats was examined in greater detail by Teitelbaum and Epstein (1962) (Epstein and Teitelbaum, 1964). They observed that although spontaneous feeding and drinking recovered in these animals if they were maintained by intragastric tube-feeding over the critical period of adipsia and aphagia, there were permanent deficits in the regulation of water intake, since the rats failed to drink after water deprivation or in response to hypertonic saline injections. Neither do these rats drink to reductions of extracellular fluid volume (Stricker and Wolf, 1967). The spontaneous water intake that does occur in rats with lesions of the LH is due to a fortuitous relationship formed between eating and drinking; these rats suffer a salivary deficit and learn to ingest small quantities of water throughout feeding to facilitate the ingestion of dry food, as described previously. This prandial drinking can be completely suppressed by the injection of small volumes of water directly into the oropharyngeal cavity as the rat eats (Kissileff, 1969b). Rats with lesions of the LH do not drink in the absence of feeding. Thus the LH was considered to be an important integrative focus for the control of drinking to body water deficits.

The results of stimulation studies have confirmed the importance of the hypothalamus in the control of fluid

ingestion. Andersson and co-workers (Andersson, 1953; Andersson and McCann, 1955a) demonstrated that micro-injections of hypertonic saline into the anterior hypothalamus elicited drinking in the goat, as did electrical stimulation (Andersson and McCann, 1955b). In the latter case drinking persisted as long as the stimulating current was maintained. Strong and persistent drinking has also been elicited by electrical stimulation of the hypothalamus in the rat, and the critical site for the effect lies within the LH (Greer, 1955; Mogenson and Stevenson, 1966). When stimulated, animals will perform tasks previously associated with obtaining water (Andersson and Wyrwicka, 1957) and like deprivation-induced drinking, the drinking that occurs is sensitive to modulatory influences such as taste (Phillips and Mogenson, 1968). The mechanisms involved in electrically-induced drinking and its relation to the normal control of water intake have recently been reviewed (Mogenson, 1973; Teitelbaum, 1973; Valenstein, 1973). Finally, the results of electrophysiological recording studies have also confirmed the involvement of the LH in the processing of cellular and extracellular stimuli of dehydration and drinking behavior (Mogenson, 1975).

Receptor and Integrative Sites for Drinking to Water Deficits

Much research has been directed towards determining the receptors and central integrative sites involved in the regulation of water intake to changes in the intracellular and extracellular fluid compartments. The early work of Verney (1947) suggested that the receptors involved in the antidiuretic response to cellular dehydration lay within the reaches of the carotid artery, and by systematic ligation Jewell and Verney (1957) determined that the osmosensitive region was located in the anterior hypothalamus. Using microinjection techniques to introduce hypertonic solutions directly into this region of the brain of goats, Andersson and McCann (1955a) demonstrated that drinking behavior as well as antidiuresis could be elicited. However, this early work (Andersson and McCann, 1955a; 1955b) suggested some anatomical separation of effective sites for antidiuresis or drinking, and functional differences between the two populations of receptors have been reported (Andersson et al., 1967). The critical area for the antidiuretic response has been circumscribed by a variety of techniques (e.g., Sundsten and Sawyer, 1961; Joynt, 1964) to be located in the region of the supraoptic nucleus. Working independently, Blass and Epstein (1971) using rats, and Peck and Novin (1971) using rabbits, both demonstrated that the preoptic area was the site of osmoreceptors involved in the regulation of water intake; ablation of

this area attenuated drinking to peripheral injections of hypertonic saline and microinjections of hypertonic solutions into this region elicited drinking. More recent studies have confirmed these findings (Blass, 1974) and electrophysiological recording techniques have demonstrated the presence of osmosensitive cells in this region of the brain (Malmo and Mundl, 1975). There is evidence that peripheral osmoreceptors located in the pancreas (Inchina and Finkinshtein, 1965) or portal circulation (Haberich, 1968) may be involved in the control of vasopressin release, although the central pathways are unknown as well as whether they participate in the regulation of water intake. The recent work of Emmers (1973), however, suggests that osmoreceptors located in the oropharyngeal cavity may be involved in the control of drinking via input through the thalamic taste nucleus (nucleus semilunaris accessorius).

As outlined previously, the receptors involved in the regulation of ECF volume probably lie within the vasculature but the neural pathways or central integrative sites remain undetermined. However, the renin-angiotensin system participates in the regulation of ECF volume (Stricker, 1973) and it has been demonstrated that the injection of nanogram quantities of angiotensin II into the anterior diencephalon elicits drinking behavior in several species (Epstein et al., 1970; Fitzsimons, 1972). Recent evidence suggests that the most sensitive brain site for inducing water intake with

angiotensin II is the subfornical organ, projecting into the third ventricle of the brain (Simpson and Routtenberg, 1973; 1975), although angiotensin II receptors may also be located in the preoptic region (Assaf and Mogenson, 1975; Kucharczyk and Mogenson, 1975).

Since lesions of the LH attenuate drinking to osmotic and volumetric signals of dehydration (Epstein and Teitelbaum, 1964; Stricker and Wolf, 1967), it is thought that efferents from osmo- and angiotensin-receptive sites may converge on the LH. The discharge rate of LH units is increased by carotid infusions of hypertonic saline (Vincent et al., 1972) and injections of angiotensin II into the forebrain (Black et al., 1973).

Limbic and Other Brain Structures

The preceding outline emphasizes the importance of the basal diencephalon in the integration of water deficit signals and water intake regulation. However, there has been increasing recognition of the importance of other brain regions, particularly limbic structures, in the control of drinking behavior. Limbic structures form a complex interconnected system with forebrain and hindbrain components and reciprocal connections with cerebral cortex and the hypothalamus (Hall, 1975; Morgane, 1975). Thus the limbic system is in a position to integrate information from the internal and external environment and influence visceral and behavioral activities.

Harvey and Hunt (1965) first reported that lesions of the septum caused increased daily water intake in rats and increased operant responding for water. The polydipsia is not secondary to polyuria (Lubar et al., 1968). Blass and co-workers (1974) have suggested that septal lesions may produce a selective increase in reactivity to volumetric signals of dehydration mediated by angiotensin II and an exaggerated reactivity to negative and positive palatability factors has been reported for rats with lesions of the septum (Beatty and Schwartzbaum, 1967). Since stimulation of the septum reduces drinking after water deprivation (Wishart and Mogenson, 1970a), this would suggest that the septum has an inhibitory influence on water intake. This could be related to a specific satiety function (Wishart and Mogenson, 1970b), analogous to the role of the ventromedial hypothalamus in the inhibition of food intake (Stevenson, 1969; Mogenson, 1974), or may be related to the observation that septal stimulation inhibits many autonomic and behavioral responses (Mogenson, 1973). More recent electrophysiological (Miller and Mogenson, 1971) and behavioral (Sibole et al., 1971) evidence suggests that the septum may also have a facilitatory influence on hypothalamic unit activity and drinking behavior, depending on the site of stimulation (Miller and Mogenson, 1972) and level of hypothalamic activity. These results indicate that the septum may have an important modulatory influence on hypothalamic function (Mogenson, 1973).

There is evidence that the amygdala is involved in ingestive behaviors (Mogenson, 1973; Mogenson and Huang, 1973) and like the septum may have inhibitory or facilitatory effects depending on the site of ablation or stimulation (Grossman and Grossman, 1963). The amygdala may participate in the integration of gustatory and olfactory influences on ingestion (Lewinska, 1968) and it has been suggested that this structure may also be involved in short-term satiety mechanisms, monitoring feeding and drinking as they occur (Mogenson, 1973).

Other limbic structures, including the cingulate gyrus (Fisher and Coury, 1962; Robinson and Mishkin, 1968), hippocampus (Olds et al., 1969; Mogenson, 1973), and limbic midbrain elements (Mogenson and Huang, 1973) have also been implicated in the control of water intake. There is little evidence that this complex interconnected system mediates primary signals of dehydration. However, the evidence that these structures can have both facilitatory and inhibitory effects and their implication in olfactory, gustatory and memory processes has led to the view that the limbic system is involved in the integration of modulatory influences (Mogenson, 1973; Mogenson and Huang, 1973).

The role of the cerebral cortex in ingestive behavior is uncertain, although the present tendency is to consider cortical involvement in higher cognitive functions influencing intake (Huang and Mogenson, 1973; Mogenson,

1974). There is some evidence that an encephalization of the neural control of feeding and drinking has occurred with evolution (Teitelbaum, 1971; Mogenson, 1974); for example, it has been proposed that in the monkey the neural control system for water intake may lie principally in the telencephalon rather than in the diencephalon (Robinson and Mishkin, 1968; Sharpe and Myers, 1969). Cortical as well as limbic forebrain structures may be involved in the control of secondary drinking (Mogenson, 1974).

Until recently the output from proposed integrative sites for the regulation of water intake has been considered in terms of caudal projections to motor nuclei in the midbrain and hindbrain involved in basic ingestion reflexes (Mogenson and Huang, 1973). Prominent projections to the midbrain have been demonstrated from regions of the hypothalamus where eating and drinking can be elicited by electrical stimulation (Huang and Mogenson, 1972) and stimulation and ablation along these pathways and their projection areas affect ingestive behavior (Mogenson and Huang, 1973). As described below, however, rostral projections from the hypothalamus and midbrain may also play a prominent role in the regulation of water intake.

Neurochemistry of Water Intake Regulation

Recent neuroanatomical developments important for understanding brain function have been achieved through the use of histochemical techniques. These have proved of

value in demonstrating the chemical identity of known fiber pathways as well as revealing projections not observed with other methods. Combined with techniques for the micro-injection of chemicals to specific sites, these have had an important impact on the study of motivated behavior in general. This rapidly expanding area of research is already too extensive to be reviewed in detail here and the reader is referred to a recent review by Mogenson and Phillips (1975).

Histofluorescence techniques have demonstrated bundles of catecholamine-containing fibers ascending from the brain-stem which project through the basal diencephalon to thalamic, limbic, and cortical regions of the brain (Dahlström and Fuxe, 1964; Ungerstedt, 1971a). Particular attention has been directed towards a dopaminergic nigro-striatal pathway which projects through the far lateral hypothalamus. Using the neurotoxic compound, 6-hydroxy-dopamine, which causes selective damage to catecholamine neurons, Ungerstedt (1971b) has shown that damaging the nigrostriatal pathway produces aphagia and adipsia in rats and that animals appear to go through the same stages of recovery as rats with electrolytic lesions of the LH. Like rats with lesions of the LH, the drinking response to cellular and extracellular dehydration is attenuated (Smith et al., 1972; Marshal and Teitelbaum, 1973). Electrolytic lesions of the nigrostriatal pathway produce the same

effect (Oltmans and Harvey, 1972). However, reservations are necessary in interpreting the effects of damage to these ascending pathways solely in terms of specific motivational deficits since sensory and motor dysfunction and alterations in general alertness have also been observed (Ungerstedt, 1971b; Marshal and Teitelbaum, 1973; Marshal et al., 1974). Furthermore, it is unlikely that damage to the dopaminergic fibers accounts completely for the effect of lesions of the LH since damage to noradrenergic and cholinergic mechanisms has also been implicated (Smith, 1973; Mogenson and Phillips, 1975).

Chemical stimulation studies have further implicated catecholamines in the regulation of water intake, although the relationship to the lesioning experiments described above remains uncertain. Grossman (1962a) reported that the direct application of norepinephrine into the LH through implanted cannulae causes eating in the rat but may actively inhibit drinking. More recent studies have demonstrated that injection of norepinephrine into the preoptic region in rats reduces drinking to water deprivation and cellular dehydration but not extracellular dehydration (Setler, 1973). Catecholamines have also been reported to stimulate drinking, although the response is weak (Setler, 1973). Isoproterenol, a beta-adrenergic agonist, has been shown to stimulate water intake (Leibowitz, 1971), but this appears to be mediated by activation of the renin-angiotensin system (Houpt and

Epstein, 1971). The dopamine antagonist haloperidol reduces drinking to angiotensin (Fitzsimons and Setler, 1971).

Cholinergic mechanisms are also involved in the control of drinking behavior, at least in the rat. The early studies of Grossman (1962a) demonstrated that the application of crystals of acetylcholine to the LH elicits drinking. Eserine, an acetylcholine esterase inhibitor, not only potentiates the drinking response but may elicit drinking when injected alone, presumably by permitting the build-up of endogenous acetylcholine (Levitt and Boley, 1970). The cholinomimetics carbachol and muscarine stimulate drinking (Grossman, 1962a; Stein and Siefter, 1962) and prior treatment with the muscarinic blocker atropine attenuates the drinking response to cholinergic stimulation (Grossman, 1962b).

The hypothalamus is not the only cholinoceptive brain site for drinking. Fisher and Coury (1962) demonstrated that cholinergic stimulation of most limbic forebrain and midbrain structures would elicit drinking, and postulated the existence of a cholinergic limbic circuit involved in the regulation of water intake. Unfortunately, the anatomical tracing of cholinergic neurons has not progressed as rapidly as for catecholaminergic systems, and has been limited primarily to techniques for detecting the presence of acetylcholine esterase; some caution is necessary not to rely exclusively on the presence of esterase as an identifying feature of cholinergic neurons (Jacobowitz and

Palkovits, 1974).. Nevertheless, the work of Shute and Lewis (1967) (Lewis and Shute, 1967) and more recently Jacobowitz and Palkovits (1974) (Palkovits and Jacobowitz, 1974) suggests the existence of cholinergic pathways ascending from the midbrain to limbic forebrain structures and numerous cholinergic limbic interconnections.

Although these studies generated initial excitement that the neurochemical substrate for water intake regulation had been identified, it was soon appreciated that reservations were in order. First of all, cholinergic mechanisms in the rat have been implicated in other regulatory processes as well, such as temperature regulation, although likely involving separate anatomical substrates (Hulst, 1972). Secondly, it appears as somewhat of a paradox that cholinergic stimulation so readily elicits drinking behavior but the peripheral (Blass and Chapman, 1971) or central (Fisher, 1973) application of anticholinergic drugs only mildly attenuates water intake following intracellular or extracellular dehydration. Finally, the demonstration of cholinergic drinking appears to be restricted to the rat; in the cat cholinergic stimulation elicits attack or sleep (Myers and Sharpe, 1968) and in the monkey blocks both feeding and drinking (Sharpe and Myers, 1969).

In summary, stimulation and ablation studies have implicated the hypothalamus in the regulation of water intake. Early models of the neural control system for

drinking suggested that signals of dehydration, probably from receptors located in the forebrain, were integrated in the lateral hypothalamus and projected to motor nuclei in the midbrain. The demonstration of bundles of ascending cholinergic and catecholaminergic fibers projecting through the diencephalon and the role of cholinergic and catecholaminergic mechanisms in drinking behavior has emphasized the importance of considering ascending pathways in the control of ingestion as well. Finally, there has been increasing recognition of the importance of extra-hypothalamic structures in the initiation and control of drinking. The results of stimulation, ablation and electro-physiological studies suggest that limbic and cortical structures may have modulatory influences on hypothalamic function and the regulation of water intake.

1.3 The Zona Incerta

The zona incerta (ZI) is a rostral extension of the mesencephalic reticular formation, located in the subthalamus of the diencephalon dorsal to the lateral hypothalamus (Pellegrino and Cushman, 1967; Barr, 1972; see Figure 1). This area is almost invariably damaged by LH lesions which disrupt drinking behavior, but until recently has received relatively little attention either in the control of water intake or in brain function in general.

Anatomical Connections

There have been few systematic studies of the anatomical relationship of the ZI with other regions of the brain, but references to afferents to this area can be found throughout the literature. Terminal degeneration has been reported in the ZI after lesions of parietal cortex (cat: Samoilov, 1971), cingulate gyrus (rat: Domesick, 1969), central gray and adjacent tegmentum of the midbrain (rat: Chi, 1970), and regions of the pons receiving afferents from the nucleus solitarius of the medulla (rat: Norgen and Leonard, 1973). Fibers have been traced to the ZI from the globus pallidus (cat: Johnson and Clements, 1959) and electrical stimulation of the pallidum inhibits the spontaneous firing of units in the ZI (cat: Tsubokawa and Sutin, 1972). There is evidence of direct somatosensory input to this region as well; ablation of the dorsal column nuclei produces degeneration

in the ZI in rats (Lund and Webster, 1967), cats (Boivie, 1971) and hedgehogs (Schroeder et al., 1968), although this projection is minor in primates (Schroeder et al., 1968), and projections of second order trigeminal fibers to the ZI have been described (Smith, 1973). Fibers of passage which originate in brainstem reticular regions have been reported to project to forebrain sites through this area (Wolf and Dicara, 1971; Lynch et al., 1973).

Efferents from the ZI have also been described. Based on his studies of normal tissue in rats, Gurdjian (1927) described incerto-tectal and incerto-tegmental connections. More recently (Huang and Mogenson, 1972) degenerating fibers have been traced from small lesions of the ZI in rats back to midbrain tegmentum including reticular formation (see also Johnson and Clemente, 1959) and central gray, the ventral half of the superior colliculus, and the region of the subcommissural organ. Degenerating terminals were observed in the region ventral to the globus pallidus and in thalamic areas (ventromedial and reuniens nuclei). Fibers were also reported to ascend in the ZI and dorsal part of the medial forebrain bundle and, rostral to the preoptic nucleus, to turn into the diagonal band and terminate in the medial and lateral septum. Fine interconnecting fibers between the ZI and the hypothalamus have been noted (Gurdjian, 1927) and Millhouse (1973) has reported afferents to the ventromedial hypothalamus from the ZI.

As noted previously, important neuro-anatomical advances have been made through the use of histochemical techniques, particularly in the identification of pathways connecting forebrain and midbrain structures. Several of these pathways are relevant to the present discussion. Although earlier studies (Ungerstedt, 1971a; Jacobowitz and Palkovits, 1974) suggested that catecholaminergic fibers passed ventral (dorsal noradrenergic bundle) and ventral and lateral (nigrostriatal dopamine pathway) to the ZI, results using the more sensitive glyoxylic acid technique (Lindvall and Björklund, 1974; Lindvall et al., 1974) suggest more extensive passage of fibers through the subthalamic regions. These include: 1) noradrenergic fibers originating in the locus coeruleus which ascend in the dorsal tegmental bundle through the ZI and medial forebrain bundle to innervate thalamus, neocortex and hippocampus; 2) a central tegmental tract originating in pontine and medullary cell groups which passes partly through the ZI to the amygdaloid-pyriform region, neostriatum and possibly cerebral cortex; and 3) components of the dorsal longitudinal fasciculus which also pass to some extent through the medial portion of the ZI to innervate the thalamus. However, none of these catecholamine fiber bundles appears to be represented entirely within the ZI and it has been reported that catecholamine histofluorescence in the subthalamus is confined almost exclusively to these fibers of passage (Lindvall et al., 1974). One notable exception, however, is a loosely

arranged bundle of delicate, probably dopamine-containing axons, originating in the caudal thalamus and medial ZI which project into the dorsal and anterior hypothalamus, forming an intradiencephalic incerto-hypothalamic system (Lindvall et al., 1974; Björklund et al., 1975).

The techniques for tracing cholinergic fibers and their limitations were discussed earlier. Ascending acetylcholine esterase-containing fibers originating in the pars compacta of the substantia nigra and ventral tegmental area ascend extensively through the ZI, projecting to the entopeduncular nucleus, globus pallidus, olfactory tubercle, and lateral preoptic area (Shute and Lewis, 1967; Jacobowitz and Palkovits, 1974). Acetylcholine esterase-containing cell bodies are located within the ZI itself (Jacobowitz and Palkovits, 1974). Via the lateral preoptic area, cholinergic connections are made with the diagonal band and septum (Shute and Lewis, 1969), which are similar to projections described using the degeneration-tracing technique (Huang and Mogenson, 1972).

Functional Implications

As early as 1927, based on his anatomical studies in the rat, Gurdjian (1927, p. 47) proposed that the ZI was "probably a very important correlation center...concerned with sub-conscious mechanisms of a vegetative nature." Nevertheless, in terms of function, "uncertain zone" remains an apt description of this region of the brain.

Electrophysiological studies in cats indicate that units of the ZI can be driven by stimulation of sciatic (Tsubokawa and Sutin, 1972), ipsilateral or contralateral peroneal or tibial nerves (Tsubokawa and Sutin, 1968) and by somatic stimulation of the limbs (Feltz et al., 1967). Furthermore, cells in the ZI respond to proximal and distal joint movements in cats (Tsubokawa and Sutin, 1968) and man (Strupples et al., 1972). These effects are characterized by short latencies (10-20 msec) and extensive convergence and are consistent with the demonstration of projections to the ZI from dorsal column nuclei. The physiological significance of these effects remains undetermined, although the work of Lindsley and co-workers suggest that the subthalamic region, including the ZI, may play an important role in modulating sensory input and regulating the responsiveness of the midbrain tegmentum to peripheral influxes (Adey and Lindsley, 1959; Lindsley and Adey, 1961; Lindsley et al., 1967). They conclude that the "subthalamus is part of a larger non-specific system involving rather complex interactions between widespread regions of the brain which play an important role in the modulation of sensory input, attentive behavior and ultimately perception" (Lindsley et al., 1967, p. 448).

Clinically, the subthalamic region has received attention since lesions in this region of the brain have been found effective in reducing the tremor of Parkinson's disease. The usual interpretation of this effect is that

lesions disrupt afferents to the ventral lateral and ventral anterior motor nuclei of the thalamus, but a recent study (Velasco et al., 1972) suggests that the most effective region for reducing tremor is in the posterior and inferior region of the ZI. In this region low voltage electrical stimulation produced no motor or sensory responses in patients, but the simple introduction of the electrode at this site was effective in arresting the tremor.

A few other studies have implicated the ZI in the control of visceral and behavioral activities. In man, lesions of the ZI may produce a slight reduction in peripheral sympathetic tone (Kim and Umbach, 1972). In monkeys electrical stimulation of the ZI, as well as other limbic structures, elicits vocalization (Robinson, 1967). In the rat, electrodes in the ZI will support self-stimulation (Phillips, 1970). However, it would appear that there have been no detailed examinations of the specific contribution of neural elements of the ZI to any particular behavior or regulatory process.

The Zona Incerta in the Regulation of Water Intake

Recent evidence suggests that the ZI may participate in the control of drinking behavior. A survey of earlier hypothalamic lesion studies indicates that severe and long-lasting adipsia and aphagia have been associated with larger LH lesions which extend into the ZI (Epstein and Teitelbaum,

1964) and the lesions reported by Montemurro and Stevenson (1957) to cause adipsia without aphagia were located in the dorso-lateral hypothalamus and ZI. More recently Huang and Mogenson (1972) have demonstrated that drinking behavior can be elicited in rats by low current electrical stimulation of the ZI as well as the more classical sites in the LH. Unlike sites within the LH eating was not elicited by stimulation of the ZI, and since the main fiber projections from the ZI and LH drinking loci differed, it was suggested that separate neural systems mediating drinking behavior may exist in these two regions.

The anatomical evidence presented above indicates that the ZI has connections with other brain structures involved in the control of drinking behavior. In light of the demonstration of acetylcholine esterase-containing cell bodies in this subthalamic region (Jacobowitz and Palkovits, 1974), it is significant that cholinergic stimulation of the ZI elicits water intake in the rat (Hulst, 1972). It has also been reported that the ZI is the most sensitive region in the ventral diencephalon from which drinking can be elicited by the direct application of cyclic adenosine monophosphate (cAMP), postulated to be involved in events underlying synaptic transmission (Rindi et al., 1972). The drinking response to cAMP stimulation is enhanced by prior treatment with the acetylcholine esterase inhibitor eserine and is blocked by the cholinergic antagonist atropine (Sciorelli et al., 1972).

The Problem for Study

Consistent with the evidence presented above, the results of two recent reports indicate that electrolytic lesions restricted to the ZI reduce daily water intake (Walsh and Grossman, 1973; Huang and Mogenson, 1974). To further examine the role of the ZI in the regulation of water intake, the studies presented in this thesis were undertaken to characterize the effect of the lesions of the ZI on drinking behavior. The experiments are divided into six parts which appear in sequence in Sections 3.1 to 3.6. They are summarized as follows:

1. Study 1 was conducted to determine the immediate and long-term effects of lesions of the ZI on daily food and water intakes and body weight gain and to test the drinking response of lesioned rats to intracellular and extracellular dehydration.

2. Since it was observed that lesions of the ZI reduced ad libitum water intake but did not reduce the drinking response to dehydration, Study 2 was carried out to confirm that the reduced intake of water was due to a direct effect on mechanisms controlling drinking behavior and was not caused by alterations in daily water requirements or excretory mechanisms.

3. The purpose of Study 3 was to determine the effect of lesions of the ZI on secondary drinking, since the results of the previous experiments suggested that lesioned rats restricted their daily fluid intake to minimal requirements.

4. It has been suggested that secondary factors rather than responses to body water deficits are responsible for the normal pattern of water intake in animals (Section 1.1). Since the results of Study 3 suggested that

lesions of the ZI reduced daily water intake by attenuating secondary drinking, daily patterns of feeding and drinking were examined in lesioned and control rats.

5. Study 5 was conducted to determine the mechanism by which lesions of the ZI reduced ad libitum water intake and secondary drinking, whether by disrupting neural elements specifically involved in the control of drinking to secondary cues or by causing a non-specific suppression of water intake.

6. Since the results of Study 5 indicated that lesions of the ZI caused a non-specific inhibition of drinking behavior, Study 6 was carried out to investigate the nature of this inhibition. The possibility of a subtle impairment in the ability of rats to ingest fluids after lesions of the ZI was examined.

CHAPTER 2. GENERAL METHODS AND HISTOLOGICAL RESULTS

2.1 General Methods

Methods common to the experiments conducted in this study are described in this Section. Exceptions to these procedures and techniques specific to individual experiments are described in the appropriate sections of Chapter 3.

Subjects, Housing and Diets

A total of 217 male Wistar rats (9 series of 20-30 rats each) was used in the 6 studies presented in Chapter 3. These were obtained at the beginning of each experiment (body weight 150-175 g) from Woodlyn Farms (Guelph, Ontario) or BioBreeding Laboratories (Ottawa, Ontario). They were housed individually in suspended wire cages (18 x 23 x 18 cm) in a room illuminated from 7:30 AM to 7:30 PM. Room temperature was maintained at 20-21°C; relative humidity varied between 20-50%.

Throughout all experiments food and water intakes and body weight (to the nearest g) were recorded daily. Tap water was provided in 50 ml graduated centrifuge tubes fitted with glass Richter tube spouts (spout opening 8 mm in diameter). Water was changed daily and intake determined to the nearest 0.5 ml.

In most experiments the rats were maintained on a synthetic granular diet (high carbohydrate diet, Table 1) freshly prepared every 2 weeks. In one experiment (Section 3.3) a high protein version of this diet was used and was obtained by increasing the proportion of casein in the diet (Table 1). These granular diets were provided in glass jars and daily food intake was determined by weighing the food dishes to the nearest 0.5 g.

In two experiments (Sections 3.2, 3.3) rats were maintained on a liquid diet described by Epstein and Teitelbaum (1962) to meet daily water and nutritional requirements. This diet (Table 1) was prepared daily and provided in 100 ml bottles equipped with glass Richter tube spouts. Intake was determined by weighing to the nearest 0.5 g. The specific gravity of this diet was found to be 1.07 and free water content, determined by drying to constant weight, was 73%.

Stereotaxic Surgery

Standard stereotaxic techniques were used to place bilateral electrolytic lesions in the region of the zona incerta (ZI). Rats were pretreated (10 min) with Atropine Methyl Nitrate (Sigma; 0.2 mg, ip) to reduce bronchial secretion and anesthetized with sodium pentobarbital (Nembutal; 50 mg/kg body weight, ip). They were then mounted in the stereotaxic frame (David Kopf, California) with the incisor bar adjusted to level the skull, and the skull

TABLE 1. SOLID AND LIQUID DIETS USED IN EXPERIMENTS

A. *Solid Diets*

Ingredients	Percent by Weight	
	High Carbohydrate Diet	High Protein Diet
Casein	20.0	70.0
White sugar	63.7	13.7
Corn oil	10.0	10.0
Salts mix*	3.8	3.8
Vitamin mix*	2.5	2.5
Caloric Density	4.11 Cal/g	4.12 Cal/g

B. *Liquid Diet*

Ingredients	Volume (ml)
Evaporated milk (Carnation)	750
50% sucrose solution (w/v)	375
Whole eggs (9 large)	450
Poly-Vi-Sol (Mead Johnson)	1
Kaopectate (Upjohn)	90
10% formalin solution	25
	1690 ml
	(1.57 Cal/g)

* Teklad Mills, Wisconsin; formulated according to recommendations for the albino rat by the Department of Nutrition, University of Toronto.

was exposed. A stainless steel or platinum-iridium wire electrode, insulated with epoxy except for 0.5 mm at the tip and less than 0.4 mm in diameter, was positioned 2.5 mm posterior to bregma and 1.5 mm lateral to the midline. (Platinum-iridium alloy electrodes were used for the rats of series 2 in Section 3.2, of series 1 and 2 in Section 3.3, and of Section 3.6, to prevent irritative iron deposits [Rabin and Smith, 1968].) A 1 mm hole was then drilled in the skull, the electrode was lowered 7.0 mm below the surface of the cortex, and a 1 ma DC current (Lesion Maker, C.H. Stoelting Co., Ill) was passed for 10 sec between the electrode tip (anode) and an electrode clipped to the animal's ear. The procedure was repeated for the opposite side of the brain and the wound sutured. The entire surgical procedure normally took less than 15 min.

Sham lesions were made in 1/3 of the animals in each series. These rats were randomly selected and underwent all surgical manipulations, including electrode penetration, except for the actual passage of current.

Post-Operative Tests

Animals were maintained for 1-3 months following surgery. During this time they were subjected to experimental procedures designed to examine various aspects of water intake regulation and body fluid homeostasis and to characterize any deficits in rats with lesions of the ZI. The procedures and results are described in detail in Sections

3.1 to 3.6.

Histological Examination

At the conclusion of the experiments the rats were anesthetized with sodium pentobarbital (Nembutal; 60 mg/kg body weight, ip) and perfused intracardially with 40 ml of isotonic saline followed by 40 ml of 10% formalin. The brains were removed and stored in 10% formalin for 2 days. They were then sectioned on a freezing microtome at a thickness of 50 μ and every second section was mounted on gelatinized slides. The sections were stained using a modified Weil procedure to stain myelinated fibres and a detailed examination of the site and extent to the lesions was carried out using the atlases of König and Klippel (1963) and Pellegrino and Cushman (1967) as guides. The histological findings are reported in Section 2.2; rats were assigned to experimental or control groups for data analysis solely on the basis of histological determination of site and extent of the lesions.

Data Analysis

Standard statistical methods (Sokal and Rohlf, 1969) were used in evaluating the results of the experiments. Quantitative results are expressed as mean ± standard error of the mean (SEM) for each group. Differences between means were evaluated using analyses of variance. For comparisons between two means, most frequently for comparison of results

of rats with lesions of the ZI with those of sham-lesioned controls after surgery, Student's *t*-test was applied. For comparison of mean values obtained before and after surgery within groups, a *t*-test for paired data was used.

An *F*-test preceded any statistical comparison of means to examine homogeneity of variance. In the case of unequal variances (primarily in comparisons of daily water intake after lesioning since it was observed that lesions of the ZI reduced the variation between rats as well as total intake), statistical evaluation was restricted to the use of the non-parametric Wilcoxon Two-Sample Test. For all analyses a probability of less than 0.05 that the difference between means would occur by chance was used as the criterion for judging statistical significance.

2.2 Histological Results and Selection of Rats for Data Analysis

As indicated in the previous Section, rats were assigned to experimental or control groups for data analysis solely on the basis of histological determination of site and extent of the lesions. Since the same criteria for selection were applied in all the experiments, these and the hitological findings are described below.

Bilateral lesions were made in 157 of the 217 rats used in the studies and 60 were sham-lesioned. The sham-lesioned rats constituted the major control group for comparison with the experimental animals. Histological examination of the brain sections from these rats revealed electrode tracks extending to the ZI but little or no damage to this region was evident.

The criteria for selection of rats for the experimental group (ZI lesions) are illustrated in Figure 1. Seventy-eight of the 157 lesioned rats sustained bilaterally symmetrical lesions located entirely within the stippled region, as determined by comparison of brain sections with the atlases of König and Klippel (1963) and Pellegrino and Cushman (1967). A representative photomicrograph is presented in Figure 2A. These lesions were located just lateral to the mamillothalamic tract (MT) and were restricted to the medial portion of the ZI. Lesions in all animals were less than 1 mm in diameter at their widest extent.

FIGURE 1

Coronal sections through the rat brain, 0.4 mm apart, from the atlas of Pellegrino and Cushman (1967). Numbers next to the sections refer to distance in mm caudal to bregma, according to this atlas; scales are also in mm. All rats included in the experimental group for data analysis sustained bilateral lesions located within the stippled region.

FX	fornix
IC	internal capsule
LH	lateral hypothalamus
ML	medial lemniscus
MT	mamillothalamic tract
ZI	zona incerta

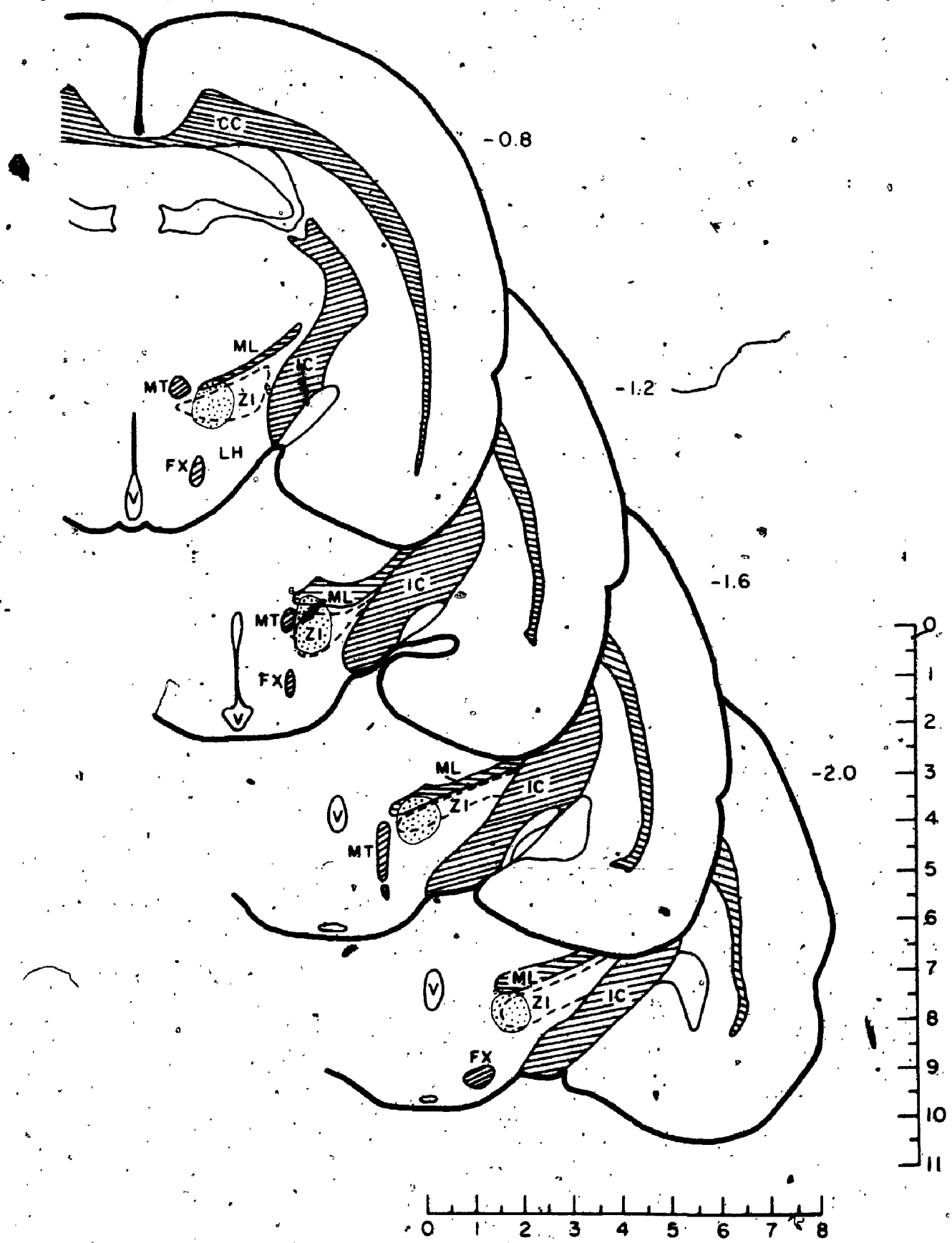

CC
-0.8
ML
IC
MT
ZI
FX
LH
V
-1.2
ML
IC
MT
ZI
FX
V
-1.6
ML
IC
ZI
V
MT
-2.0
ML
ZI
IC
V
FX
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8 9 10 11

FIGURE 2

Photomicrographs of coronal brain sections, illustrating the location and size of lesions (indicated by arrows) at their widest extent.

A. Section from a rat which received bilateral lesions of the zona incerta.

B. Section from a rat which received control lesions dorsal to the zona incerta.


A
2 mm


B
2 mm

Since the rats were not sacrificed until at least one month after surgery, some shrinkage of the ablations likely occurred (Wolf and DiCara, 1969); however, the small size of the lesions is in agreement with the small lesioning current passed (1 ma, 10 sec).

A slight dorsal bias in lesion site was used in the selection of the experimental group to minimize damage to the lateral hypothalamus (LH), which has also been implicated in the control of water intake (Section 1.1). Because of this, partial damage to the medial lemniscus was observed in some animals and more posterior lesions often encroached on the pallidofugal fibres of the H_1 field of Forel. Although damage to these regions was minimal and not consistently observed, a second group of 32 rats was selected from the lesioned animals for data analysis, to control for the effect of lesions of these dorsal structures. These rats sustained bilateral lesions centered on the medial lemniscus which extended dorsally into the thalamus and ventrally into the H_1 field of Forel, but produced only minimal or no damage to the ZI. A representative photomicrograph is presented in Figure 2B. Throughout the experiments, these rats are referred to as dorsal lesioned or control lesioned rats.

Finally, 47 rats receiving electrolytic lesions were not included in the data analysis. Lesions in these rats were asymmetrical and included damage to other nearby structures such as the MT and LH. Since these lesions also

included at least partial damage to the ZI, these rats could not be assigned to control lesion groups.

CHAPTER 3. THE EXPERIMENTS

The experiments conducted in this study are presented in the following sections. They have been divided into six parts and the results of each are described and discussed separately. A general discussion and summary of the results is presented in Chapter 4. Histological results, because they were similar for all experiments and were used as criteria for selecting animals for data analysis, have been described in detail in Section 2.2.

3.1 Daily Water Intake and Drinking to Body Water Deficits in Rats with Lesions of the Zona Incerta

The evidence reviewed in the Introduction indicates that the zona incerta (ZI) may participate in the control of drinking behavior of rats. Drinking can be elicited by electrical (Huang and Mogenson, 1972) and chemical (Rindi et al., 1972) stimulation of the ZI and the results of two recent reports indicate that lesions of the ZI reduce daily water intake (Walsh and Grossman, 1973; Huang and Mogenson, 1974). The purpose of the experiments presented here was to further examine the immediate and long-term effects of lesions of the ZI on ad libitum water intake. In addition, an attempt was made to characterize the reduction in daily

water intake by testing the drinking responses of lesioned and control rats to reductions of intracellular and extracellular fluid volumes. Lesions of the adjacent lateral hypothalamus (LH), which almost invariably encroach on the ZI, attenuate these regulatory responses (Epstein and Teitelbaum, 1964; Stricker and Wolf, 1967), and it has been suggested that lesions of the ZI may do so also (Walsh and Grossman, 1973).

METHODS

Two series of 24 rats each were studied consecutively, and initially received identical treatment. Following a one-week stabilization period, baseline measures of food and water intakes and body weight were obtained for one week. Then bilateral electrolytic lesions were placed in the region of the ZI; 8 of the rats of each series were randomly selected to receive sham lesions. The effects of the lesions on ad libitum food and water intakes and body weight were measured for six days after surgery.

Series 1: Drinking in Response to Body Water Deficits

The rats of Series 1 were tested for impairment in the drinking response to body water deficits. The tests are described below and include food deprivation (FD), water deprivation (WD), cellular dehydration (CD), and extracellular dehydration (ECD). The order of the tests for all

animals in Series 1 was as follows:

FD - WD - CD - ECD - WD - ECD - CD - WD - FD

These tests were extended over a two-month period and at least three days separated each test. When the test series was completed the animals were maintained for an additional week to determine any changes in ad libitum food and water intakes. The rats were then sacrificed and site and extent of the lesions determined histologically.

a. *Food deprivation.* Rats with lesions of the LH drink only when eating dry food and fail to drink when food is absent; this is indicative of disruption of all regulatory drinking responses except those related to specific oropharyngeal cues (Epstein and Teitelbaum, 1964). To test for this phenomenon in rats with lesions of the ZI, on two occasions the animals were food-deprived for 24 hours beginning at 9:00 AM. Water was freely available throughout the deprivation and water intake during this period was determined and compared with that of the previous 24 hours when food was present.

b. *Water deprivation.* The simplest way to induce an animal to drink is by water-depriving it. On three occasions the rats of Series 1 were deprived of water: for 12 hours on the first test, for 24 hours on the second test, and for 18 hours on the third test. Water was removed at 9:00 PM, 9:00 AM, and 3:00 PM, respectively, and returned the next day at 9:00 AM. Water intake was measured for the

one-hour period following deprivation. In all cases, food was available throughout the deprivation periods, but not during the one-hour test of water intake.

c. Cellular dehydration. A reduction in intracellular fluid reliably induces drinking in intact animals and is considered to be a primary stimulus regulating water intake to need. Lesions of the LH attenuate the drinking response to cellular dehydration (Epstein and Teitelbaum, 1964). To determine whether or not lesions of the ZI have a similar effect, cellular dehydration was induced by the injection of hypertonic saline and the drinking response observed. Rats were randomly assigned to two groups: one group received intraperitoneal injections of 2M NaCl, at a dose of 1 ml/kg body weight; the other group was injected with equivalent volumes of isotonic saline to control for injection procedures. Latency to drink following injection and one-hour water intake were determined. Food was not available during this period. The next day the treatments were reversed; animals which had received control injections previously received hypertonic saline and vice versa. As noted above, this entire sequence was repeated twice for each animal.

d. Extracellular dehydration. To determine whether or not lesions of the ZI disrupt the drinking to extracellular dehydration, as do lesions of the LH (Stricker and Wolf, 1967), polyethylene glycol (PG, mol wt = 20,000)

dissolved in isotonic saline (20% w/v) was injected subcutaneously in the rats at a dose of 20 ml/kg body weight, one-half of the dose to each flank. This procedure induces hypovolemia by sequestering isotonic fluid at the point of injection. As a control procedure, an equivalent volume of the saline vehicle was administered. The order of treatment, control or PG, with two days between injections, was randomized as in the cellular dehydration tests. Water was freely available and intake determined hourly for ten hours after injection. Food was not available during this period. This test procedure was repeated twice.

Series 2: Water Intake following Water Deprivation

The second series of rats was used to study further the effects of lesions of the ZI on the drinking elicited by water deprivation since the results from the first series were inconclusive. Beginning seven days after surgery, the rats were subjected to periods of water deprivation lasting 0, 6, 12, 24, and 48 hours. Food was available ad libitum throughout the deprivations. All animals were subjected to each of the deprivations, but the order of treatment was randomized such that for any given test session all deprivations were represented. Within a test session, the deprivations were arranged so that water was returned to all animals at 7:00 AM, the end of the dark period of the day-night cycle. The rats were weighed, food was removed from

the cages, and fresh water made available. Water intake over the next hour was determined. Deprivations were separated by a minimum of three days. At the end of the tests the rats were sacrificed and site of the lesions determined histologically.

RESULTS

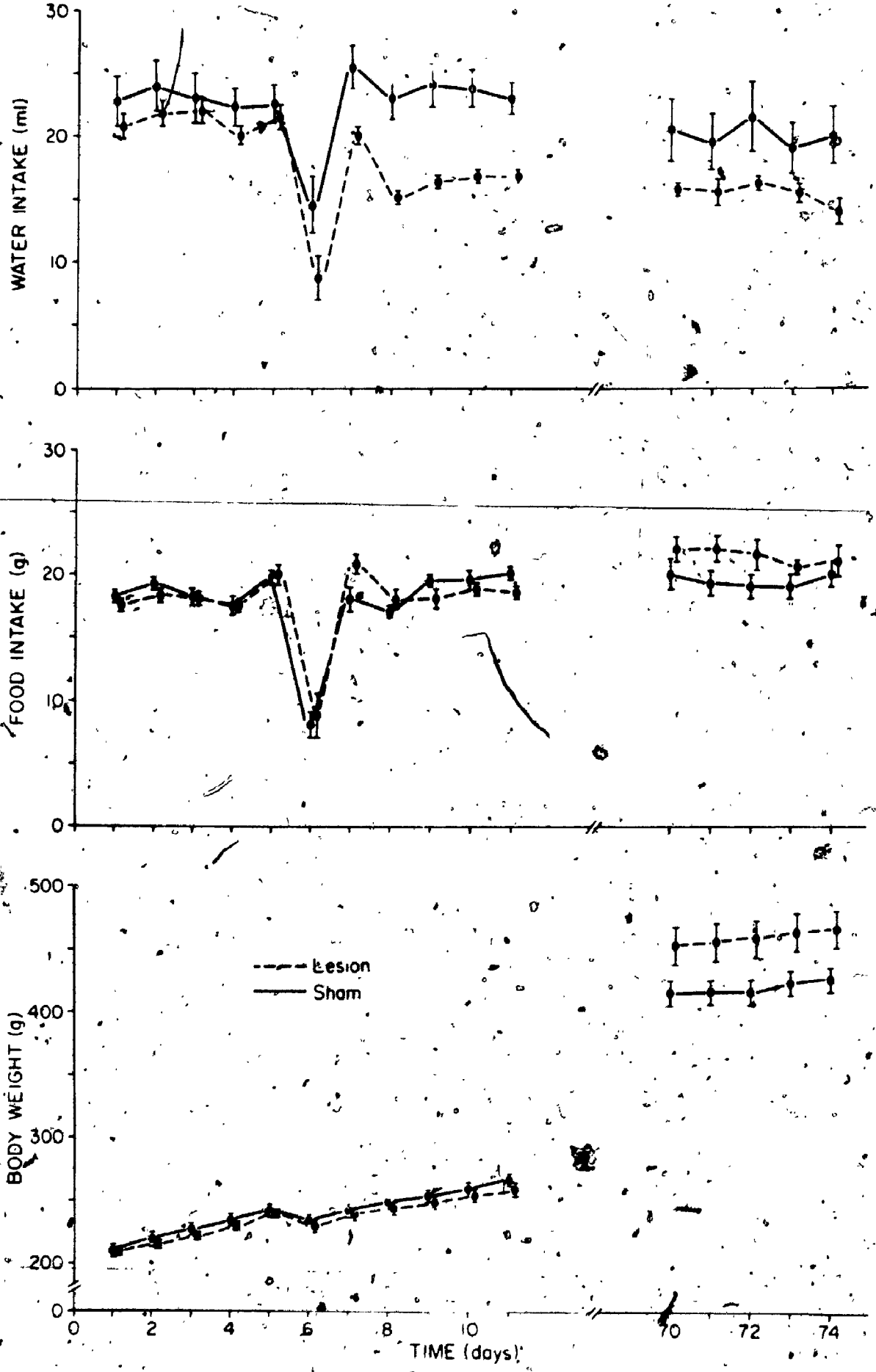
Ad Libitum Intakes and Weight Gain

The immediate effects of lesions of the ZI on mean daily food and water intakes and weight gain are presented in Figure 3. Since these effects were similar for both series, the data are combined. The results obtained from animals of Series 1 two months after surgery are also presented in Figure 3.

Lesions of the ZI caused a reduction in mean daily water intake. From the second post-operative day onward the lesioned animals consumed only about 75% of their pre-operative water intake ($P < 0.01$). They were still drinking less than controls two months later ($P < 0.05$) and showed no sign of recovery to pre-operative levels of water intake. Furthermore, there was a significant reduction in the variance of the water intake within the lesioned group after surgery. The variance of the group receiving lesions of the ZI was less after surgery than before ($P < 0.01$) and much less than controls immediately after surgery ($P < 0.01$) and two months later ($P < 0.01$). The data indicate that

FIGURE 3

Daily water and food intakes and body weight (means ± SEM) for rats with lesions of the zona incerta (ZI) and sham-lesioned controls. Surgery was performed on Day 6. For Days 1-11, the results of Series 1 and 2 are combined (N = 16 for each group). Results are also presented for the rats of Series 1 for Days 70-74 (N = 8 for each group).


WATER INTAKE (ml)
30
20
10
0
FOOD INTAKE (g)
30
20
10
0
BODY WEIGHT (g)
500
400
300
200
0
Lesion
Sham
0
2
4
6
8
10
70
72
74
TIME (days)

following lesions of the ZI, water intake tended to approach a rather constant level regardless of prelesion intake. For example, rat 7373 drank 27-28 ml of water per day before surgery, but only 16-17 ml after lesioning, whereas lesions of the ZI produced no change in water intake in rat 7249, which ingested 16-17 ml before surgery. Rats sustaining dorsal lesions, causing only minor damage to the ZI, were similar to controls and did not exhibit the reduction in water intake (N = 4, 19.9 ± 1.4 ml/day before lesioning, 19.7 ± 2.3 ml/day after).

Lesions of the ZI had no significant effect on food intake either immediately after surgery or during the two-month post-operative period. There were no immediate effects of the lesions on body weight or daily weight gain. By the end of the two-month period the lesioned rats of Series 1 had gained slightly more weight than controls but this was not statistically significant ($P > 0.10$). This increased weight gain was not observed in the lesioned rats of Series 2 or in subsequent experiments. There were no indications of gross motor impairment and from outward appearance lesioned rats were indistinguishable from controls.

Food Deprivation

In contrast to rats with lesions of the LH (Epstein and Teitelbaum, 1964), rats with lesions of the ZI drank water when food was not available. The results for the two

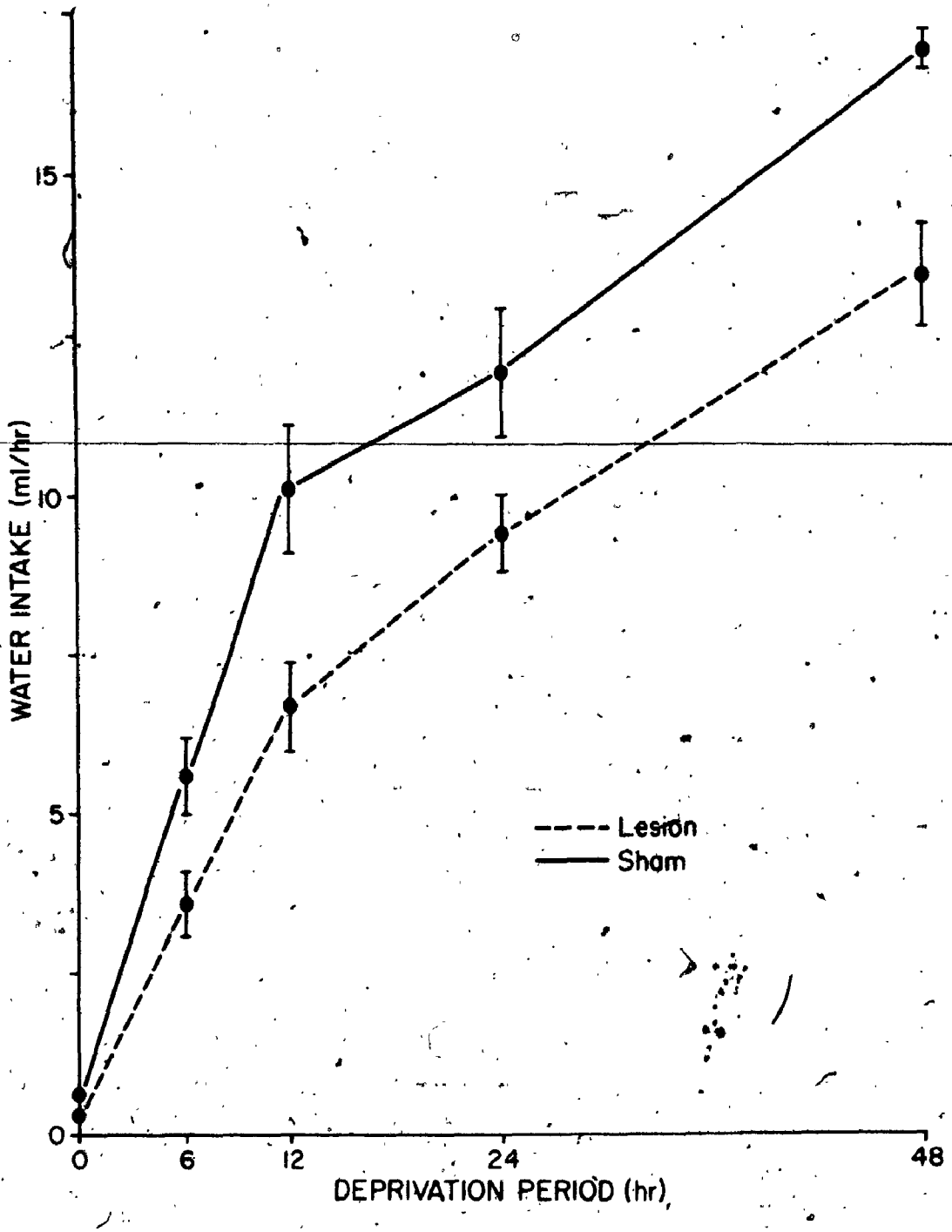
food deprivation tests were similar and are combined. For the 24 hours just prior to removing their food the lesioned (N = 8) and sham-lesioned control rats (N = 8) consumed 16.5 ± 0.5 ml and 22.0 ± 2.2 ml of water respectively ($P < 0.01$). During the 24 hours of food deprivation they drank 9.5 ± 1.8 ml and 14.4 ± 2.8 ml, respectively ($P > 0.10$).

Water Deprivation

Rats with lesions of the ZI drank following water deprivation. The results from Series 1 suggested that lesioned rats drank slightly less than controls, although the difference was significant only following the 12-hour deprivation period ($P < 0.01$). These results were somewhat inconclusive, however, since water intake in both lesioned and sham-lesioned rats did not increase with longer periods of deprivation, perhaps due to the temporal sequence and spacing of the tests. The water deprivation tests were repeated under more carefully controlled conditions in Series 2 and the results are presented in Figure 4. Both groups drank following all periods of deprivation, consuming more water following the longer deprivations, but rats with lesions of the ZI consistently drank less than controls ($P < 0.01$). However, the difference in intake was similar for all deprivation periods (2.0, 3.4, 2.5 and 3.5 ml) and was comparable to that observed in Series 1 (3.1, 2.2 and 2.0 ml). This indicates that the deficit in water intake

FIGURE 4

Water intake (mean ± SEM) of rats with lesions of the zona incerta (ZI) and sham-lesioned controls (N = 8 for each group) in the hour following periods of water deprivation.


15
10
5
0
WATER INTAKE (ml/hr)
0
6
12
24
48
DEPRIVATION PERIOD (hr)
Lesion
Sham

did not vary with the state of dehydration.

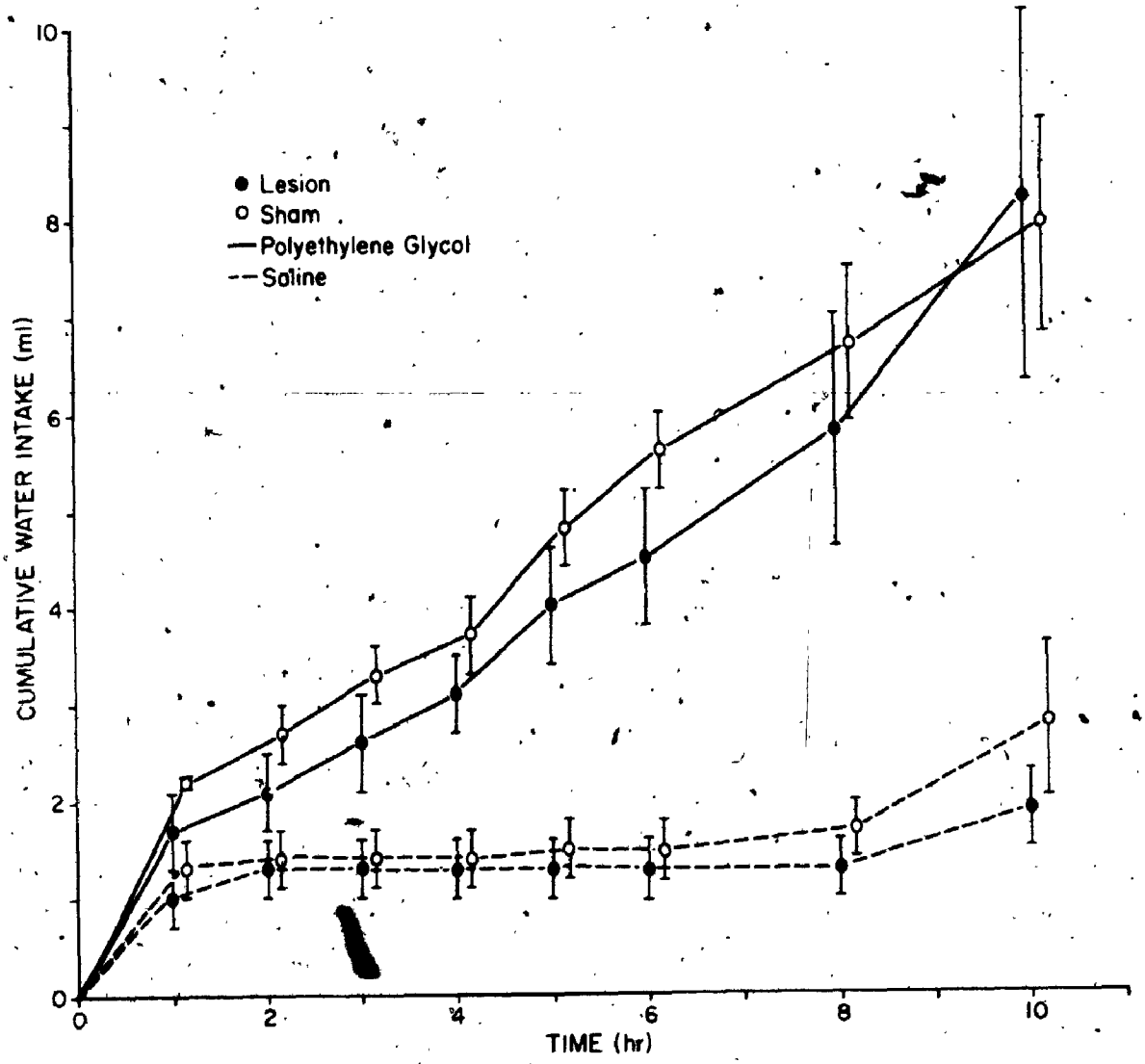
Cellular Dehydration

Rats with lesions of the ZI and sham-lesioned rats drank following the injection of hypertonic saline. Water intake in response to cellular dehydration was calculated for each rat as the difference in intake following the injection of hypertonic saline and that following the control injection of isotonic saline. Intakes for the two groups for both tests were significantly greater than zero ($P < 0.01$) but not different from each other. Based on average values for each rat combined for the two tests, lesioned rats drank 1.53 ± 0.32 ml to the osmotic stress in comparison to 1.24 ± 0.34 ml for the controls. These intakes are relatively small but are within the range expected for the mild osmotic challenge used and emphasize that lesions of the ZI do not reduce responsiveness to even small osmotic changes. There was no difference between groups in the latency to drink following hypertonic saline injections.

There was one noteworthy difference in the drinking response of the two groups under these test procedures. Following the control injections of isotonic saline for the first test lesioned and control rats drank similar volumes of water (0.9 ± 0.3 ml for each group) which were not significantly different from each other or from normal one-hour water intakes for these rats at the time of testing.

FIGURE 5

Cumulative water intake (mean ± SEM) induced by subcutaneous injections of polyethylene glycol and control injections of isotonic saline in rats with lesions of the zona incerta (ZI) and sham-lesioned controls (N = 8 for each group). Injections were made at time 0.


Lesion
Sham
Polyethylene Glycol
Saline
CUMULATIVE WATER INTAKE (ml)
TIME (hr)
0
2
4
6
8
10

However, on the second test the control rats consumed a significantly greater amount of water to the control injections (2.3 ± 0.4 ml) than the rats with lesions of the ZI (0.9 ± 0.3 ml, $P < 0.02$).

Extracellular Dehydration

Lesions of the ZI did not reduce water intake following subcutaneous injections of polyethylene glycol (Figure 5). The results for the two tests were similar and were combined. There was no difference between groups in latency to drink or total amount of water consumed as shown in Figure 5.

DISCUSSION

Bilateral lesions of the medial portion of the ZI caused a significant reduction in ad libitum water intake, confirming previous observations (Walsh and Grossman, 1973; Huang and Mogenson, 1974). The hypodipsia was long-lasting and characterized by a level of water intake that showed little variation between rats regardless of prelesion water intake. In contrast to the effects of lesions of the LH (Epstein and Teitelbaum, 1964), there was neither an initial period of adipsia nor a reduction in food intake or body weight gain. Also unlike rats with lesions of the LH, rats with lesions of the ZI continued to ingest water when food-deprived, indicating that they were not solely dependent on feeding-related cues for fluid ingestion.

The reduction in ad libitum water intake after lesioning cannot be attributed to a failure to respond to water deficit signals. Rats with lesions of the ZI drank in response to injections of hypertonic saline which reduce intracellular fluid volume and to injections of hyperoncotic colloid which sequester extracellular fluid. Finally, rats with lesions of the ZI drank following water deprivation and the volume ingested was proportional to the length of deprivation. Although lesioned rats drank slightly less than controls after water deprivation, the difference in intakes between the two groups did not vary with the length of deprivation, further indicating that the deficit was not related to state of dehydration. Consistent with these results, data presented by Wolf (1971) in a study of lesion effects on Na^+ appetite demonstrate that lesions which damage the ZI do not influence water intake when extracellular fluid volume is reduced by Na^+ depletion. It is concluded that the medial portion of the ZI is not necessary for drinking in response to water deficit signals and that the hypodipsia observed after lesioning cannot be attributed to loss of these regulatory mechanisms.

The deficit in drinking behavior reported by Walsh and Grossman (1973), who also observed a reduction in ad libitum water intake after lesions of the ZI, does not appear to be the same as that observed in this study. Since their lesioned rats failed to drink in the absence of food, even

on the third day of deprivation, they concluded that there was a severe deficit in regulatory drinking. Recently, Walsh and Grossman (1974) have reported that drinking in response to cellular dehydration is attenuated in these animals. However, their lesions were larger and more anterior than those presented here and may have extended into more ventral regions of the diencephalon to include other areas involved in the control of drinking. In the more posterior regions of the ZI where the present lesions were located, Walsh and Grossman (1973) were unable to separate effects on food and water intake, again perhaps due to the size of the lesions and extension into the LH.

It is significant that lesions of the ZI do not attenuate drinking to signals of water deficit but do influence ad libitum water intake. In the past major emphasis has been placed on the role of regulatory drinking responses to water deficits in maintaining body fluid homeostasis, and the disruption of fluid ingestion following ablation of various brain structures has been attributed to effects on neural elements subserving these regulatory responses. However, stimuli not directly related to water needs for fluid balance, termed secondary factors by Fitzsimons (1972), can also initiate and modulate drinking behavior and may actually determine the daily pattern and amount of water ingested by an animal. Lesions of the ZI may reduce ad libitum water intake by attenuating secondary drinking without affecting drinking to deficit signals.

There is evidence that rats normally consume more water than they actually need for fluid balance (Dicker and Nunn, 1957) and the reduced and less variable intake observed after lesions of the ZI may indicate that fluid ingestion was being more precisely regulated to need. It is also noteworthy that although control rats were observed to drink a significant amount of water solely in response to a control injection, perhaps as the results of arousal and repeated testing (Mineka and Seligman, 1975), this was not observed in rats with lesions of the ZI.

Since electrical and chemical stimulation of the ZI elicit drinking behavior, the lesion results have been interpreted as an effect on water intake mechanisms. However, it does not necessarily follow that lesions and stimulation results are due to a direct effect on the same neural elements. Before postulating the role of the ZI in drinking to non-deficit cues, it is necessary to verify that the results of the lesions are due to a primary effect on drinking behavior. The next section presents the results of several experiments conducted to test the hypothesis that the reduction in drinking is secondary to changes in other aspects of water balance, such as excretory rate or daily water requirements.

3.2 Water Balance and Running-Wheel Activity in Rats with Lesions of the Zona Incerta

The results presented in Section 3.1 confirm that lesions of the ZI reduce ad libitum water intake, but indicate that the hypodipsia cannot be attributed to a deficit in the drinking response to signals of dehydration. The present experiments were carried out to determine whether the hypodipsia was actually due to a disruption of neural elements involved in the control of drinking behavior or was secondary to changes in other regulatory mechanisms maintaining body fluid homeostasis.

The first series of experiments examined several aspects of water balance in rats with lesions of the ZI to determine whether the reduction in water intake could be related to deficits in excretory mechanisms or to reductions in daily water requirements. It was also of interest to investigate whether alterations in water intake and water losses were complementary, enabling lesioned rats to remain in water balance, since it was observed that rats with lesions of the ZI continue to eat and gain weight normally for long periods after surgery (Section 3.1).

The results of these experiments indicated that the reduced water intake after lesioning was related to a direct effect on drinking behavior. Since it has been suggested that fibers projecting through the ZI comprise part of the ascending pathway of the reticular arousal system (Shute

and Lewis, 1967), a final experiment was carried out to determine whether the deficit after lesioning merely reflected a non-specific depression of behavior. The effect of lesions of the ZI on a second spontaneously-occurring behavior, running-wheel activity, was determined and compared with the effect on water intake.

METHODS

Series 1: Water Exchange when Water Available Ad Libitum

The volume and osmolality of urine excreted by rats with lesions of the ZI were measured to determine whether changes in urinary water losses accompany the reduction in ad libitum water intake. To examine the effect of lesions and the resulting change in water turnover on body fluids, serum samples were obtained and analyzed one month after surgery.

Twenty-three rats were provided with food and water ad libitum. On the fifth day of the experiment, collecting funnels equipped with wire grids to separate feces were mounted beneath the cages and urine collected in graduated cylinders. Twenty-four hour urine volume was recorded daily for the next six days. Then bilateral electrolytic lesions were placed in the region of the ZI of 16 of the rats; the remaining seven served as sham-lesioned controls. Daily food and water intake, body weight and urine volume were measured for seven days post-operatively and urine osmolality was determined for the last three days of this

period (Osmometer, Advanced Instruments, Inc., Mass.).

Food and water intake and body weight were monitored continuously for another 3 1/2 weeks and then blood samples were drawn to determine the effect of the lesions on serum osmolality and electrolyte level. The rats were lightly anesthetized with ether. The tail of each rat was wiped with 70% ethanol, placed in warm water for one minute, dried with clean gauze and severed 1 cm above the tip. The first few drops of blood were discarded and about 2 ml collected. At the same time a small amount of blood was collected in a micro-hematocrit tube (Propper Mfg. Co., New York). The wound was cauterized and the animal allowed to recover. The samples were centrifuged, then the serum was drawn off and analyzed for osmolality and Na^+ and K^+ concentration (flame photometer, Instrumentation Laboratory Inc., Mass.). One week later the rats were sacrificed and the site and extent of the lesions determined histologically.

Series 2: Water Exchange with Fluid Intake Matched to Controls

This experiment examined the effect of lesions of the ZI on water balance in rats in which fluid intake was matched to that of controls. Under these conditions deficits in water excretory mechanisms or reduced water requirements (including reduced evaporative losses) after lesioning should be reflected as decreased or increased volumes of

urine output, respectively. Since lesions of the ZI do not reduce daily food intake (Section 3.1), to match water intake twenty-four rats were maintained on a liquid diet (Section 2.1) designed to meet all daily water and nutritional requirements. For the first part of the experiment water was also available. Fourteen days after the beginning of the experiment the rats were lesioned and seven of the rats were randomly selected for sham-lesioning. Seven days after the surgery the water tubes were removed from the cages and the animals were maintained for a further six days provided only with the liquid diet. From the fourth to the sixth day of this period, urine was collected as in the first experiment and analyzed for volume, osmolality, and Na^+ and K^+ concentration. The rats were then placed on the solid diet used in the first experiment as well as provided with water ad libitum to confirm that lesions in this series also produced the hypodipsia observed in the previous studies. Six days later the rats were sacrificed and lesion site determined.

Series 3: Running-Wheel Activity and Excretion of a Gastric Water Load

Twenty rats were housed individually in cages equipped with activity wheels (Wahmann Mfg. Co., Maryland). Access to the running wheel, food and water were continuously available. Rectal temperature (Yellow Springs Instrument Co. telethermometer, thermistor probe inserted 5 cm beyond

the anal orifice) was measured daily throughout the experiment. The rats were allowed one week to adapt to the activity cages. During the following week baseline measures of food and water intake, body weight, activity and rectal temperature were recorded. Then 14 of the rats received electrolytic lesions in the region of the ZI; the remaining six were sham-lesioned. Food and water intake, body weight, running-wheel activity and rectal temperature were measured for three weeks post-operatively.

At the end of this period the rats were transferred to suspended wire cages and allowed to adapt for one week. The excretion of a gastric water load was then examined, to determine whether or not the reduced drinking in rats with lesions of the ZI could be attributed to a reduction in the ability to excrete fluid. The night before the water load test the rats were food-deprived to reduce defecation during the test; water was available ad libitum. The next morning the water tubes were removed and in each animal the bladder was emptied by applying supra-pubic pressure and the rat weighed to the nearest 0.5 g. Distilled water at room temperature was then administered by stomach tube in a volume equal to 3% of the body weight. The rats were re-weighed and returned to their cages. Collecting funnels equipped with wire grids to separate feces were placed beneath the cages and urine collected in graduated cylinders. Body weight and urine volume (defecation was negligible)

were then determined 1/4, 1/2, 1, 2, 3, and 4 hours after the water load. At the end of this experiment the rats were sacrificed and site and extent of the lesions were determined.

RESULTS

Water Exchange with Water Available Ad Libitum

As observed in previous experiments and presented in Table 2, lesions of the ZI significantly reduced ad libitum water intake. Sham lesions or control lesions dorsal to the ZI did not reduce daily water intake nor did any of the lesions significantly alter daily food intake or body weight gain. Accompanying the 30% reduction in water intake in rats with lesions of the ZI was a 30% reduction in urine volume, and a significantly higher urine concentration (Table 2). These two changes were complementary in that the total solute load excreted did not differ significantly between groups (determined as the product of urine volume and osmolality for each rat, $P > 0.20$).

Table 3 presents the results of the blood serum analyses one month after surgery. There were no significant differences between lesioned and control rats in hematocrit, serum Na^+ or K^+ concentrations or serum osmolality.

TABLE 2. DAILY WATER INTAKE, URINE VOLUME AND URINE OSMOLALITY BEFORE AND AFTER LESIONS.

Group	Water Intake (ml/day)		Urine Volume (ml/day)		Urine Osmolality (mOsm/kg)
	Before	After	Before	After	After
Sham Lesions (N = 7) :	27.2 ± 2.9	25.1 ± 2.2	13.9 ± 2.6	13.1 ± 2.2	1533 ± 168
Dorsal Lesions (N = 3)	29.3 ± 5.2	26.4 ± 7.8	16.0 ± 4.4	15.7 ± 6.6	1824 ± 550
ZI Lesions (N = 8)	24.6 ± 1.5	16.8 ± 0.7*†	11.4 ± 1.2	8.0 ± 0.6*†	2140 ± 150*

Values are means SEM for each group, calculated from the average daily value for each rat for the periods before and after surgery. "Before" refers to the 3 days immediately preceding surgery. "After" refers to the 7 days following surgery, commencing on the day after surgery; urine osmolality was determined for the last 3 days of this period.

* significantly different from the value for the sham lesion group, $P < 0.05$.

† significantly different from "before" value, $P < 0.05$.

TABLE 3. HEMATOCRIT AND SERUM Na^+, K^+ AND OSMOLALITY ONE MONTH AFTER SURGERY

Group	Hematocrit (percent)	Serum Na^+ (mEq/l)	Serum K^+ (mEq/l)	Serum Osmolality (mOsm/kg)
Sham Lesions (N = 7)	43.7 ± 1.3	141.0 ± 0.7	5.8 ± 0.2	305.4 ± 1.5
ZI Lesions (N = 8)	46.1 ± 1.2	141.9 ± 0.6	5.6 ± 0.2	304.1 ± 1.9

Values are means ± SEM.

Water Exchange with Fluid Intake Matched to Controls

During the 14-day pre-operative period rats readily consumed the liquid diet and continued to gain weight. That the diet was adequate to meet the animals' water needs is evident in the dilute urine excreted (Table 4) and the fact that when the diet was the only source of fluid available there were no significant changes in the rate of weight gain.

Lesions of the ZI had no significant effect on the ingestion of the liquid diet (Table 4); thus rats with lesions of the ZI and sham-lesioned controls were consuming similar volumes of fluid. Under these conditions there were no differences between lesioned and control rats in urine volume or osmolality or the excretion of electrolytes (Table 4). Neither were there any significant differences in body weight gain. When these rats were switched to the regular solid diet the previously reported hypodipsia was observed, with no concomitant differences in food intake or weight gain (sham-lesioned rats: 37.4 ± 2.2 ml water/day; ZI-lesioned rats: 24.1 ± 0.7 ml water/day; $P < 0.01$).

Excretion of a Gastric Water Load

There was no significant difference between rats with lesions of the ZI and control rats in the volume of urine excreted following an intragastric water load (Figure 6). Weight loss with time, which reflects in part total water loss, also did not differ between groups.

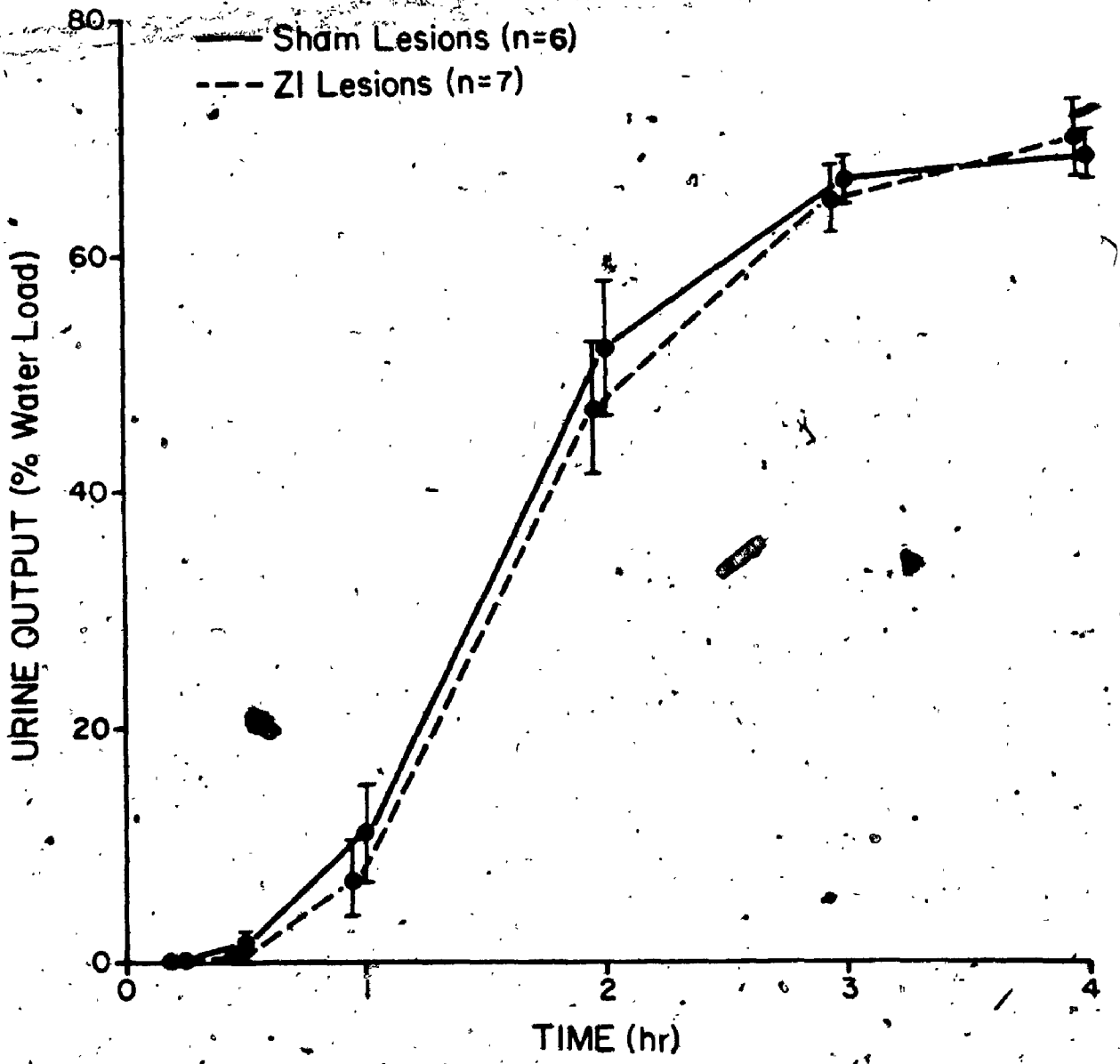
TABLE 4. URINE AND ELECTROLYTE EXCRETION BY RATS MAINTAINED ON LIQUID DIET WITH WATER NOT AVAILABLE

Group	Diet Intake (ml/day)	Urine Volume (ml/day)	Urine Osmolality (mOsm/kg)	Na^+ Output (mEq/day)	K^+ Output (mEq/day)
Sham Lesions (N = 7)	64.4 ± 2.5	28.1 ± 1.9	620 ± 16	2.09 ± 0.13	1.56 ± 0.09
ZI Lesions (N = 7)	65.9 ± 2.5	27.9 ± 1.8	610 ± 13	2.03 ± 0.12	1.57 ± 0.08

Values are means ± SEM calculated from the average of 3 days for each rat.

FIGURE 6

Cumulative urine excretion (volume as a percent of water load, mean ± SEM) after an intragastric water load (3% of body weight) in rats with lesions of the zona incerta (ZI) and sham-lesioned controls.


Sham Lesions (n=6)
ZI Lesions (n=7)
URINE OUTPUT (% Water Load)
80
60
40
20
0
0
1
2
3
4
TIME (hr)

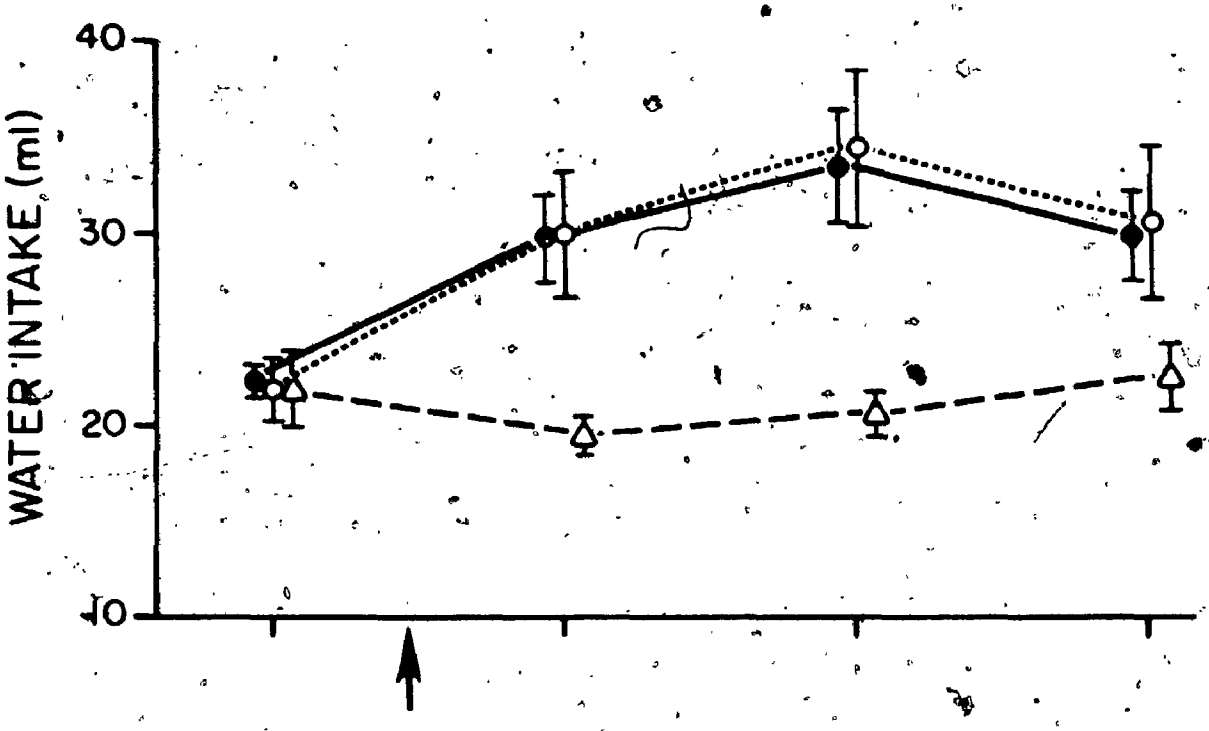
Running-Wheel Activity

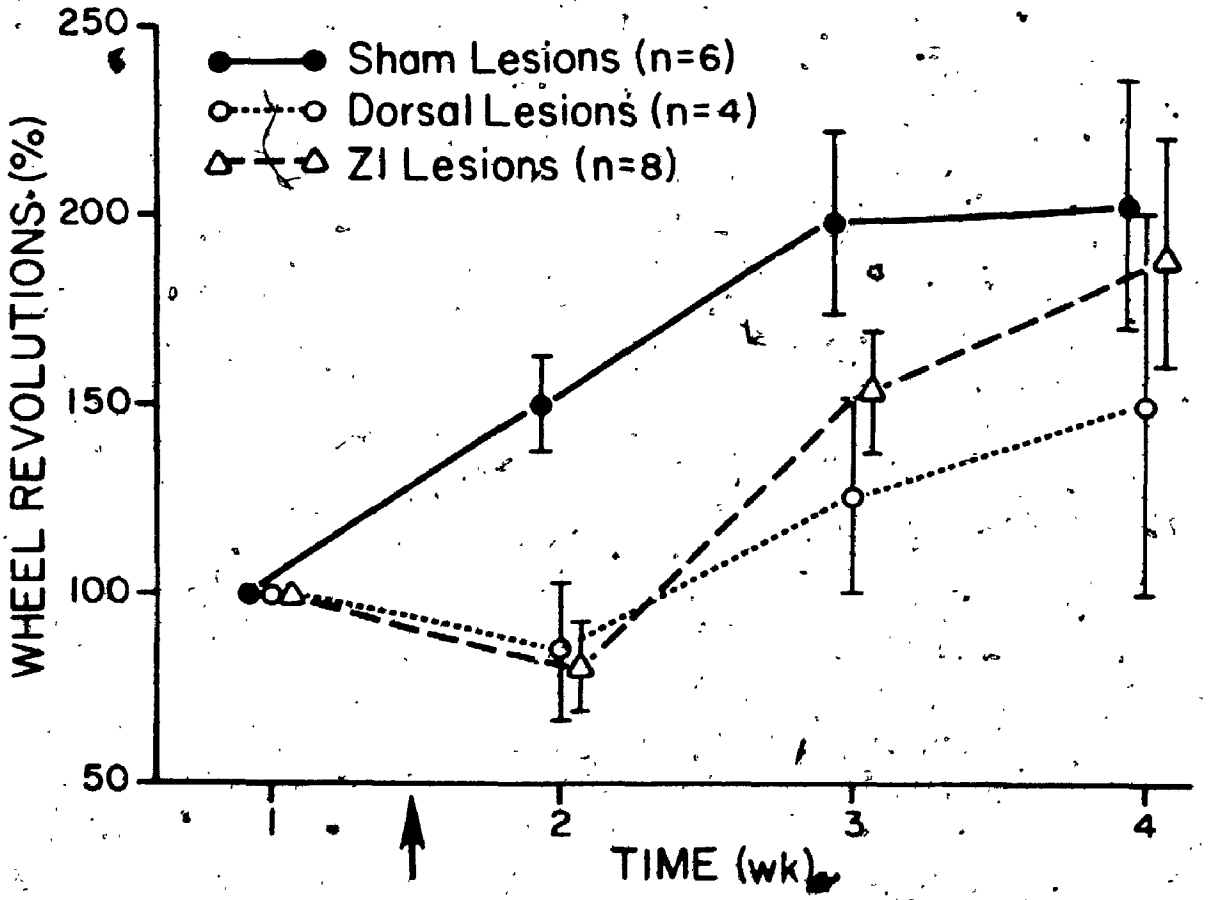
The effects of lesions on water intake and wheel-running are presented in Figure 7. Mean water intakes were determined for each animal for each of four 7-day periods extending from the week before surgery to three weeks after. Intakes for the first two post-operative days were not included to avoid immediate surgical effects. Mean activity levels were calculated for the same periods, but because of the wide variation in the absolute number of wheel revolutions between animals, it was necessary to normalize the data; the pre-operative mean activity level of each rat was taken as 100 and subsequent activity was expressed as a percentage of pre-operative level.

Lesions of the ZI reduced both spontaneous water intake and running-wheel activity (Figure 7). Control rats throughout the two-week period and lesioned rats before surgery exhibited a gradual increase in both measures but lesions of the ZI disrupted this trend. However, the effects on wheel activity and drinking are different. First, during the week following surgery, there was no significant correlation between depression of activity and post-operative water intake ($r = 0.40$, $P > 0.2$) or reduction in water intake ($r = 0.10$, $P > 0.5$) in rats with lesions of the ZI. Secondly, rats with lesions of the ZI showed recovery of wheel-running to control levels by the third post-operative week. This was not observed for water intake. Drinking in

FIGURE 7

Average daily water intake and running-wheel activity (mean ± SEM) in rats with lesions of the zona incerta (ZI), lesions dorsal to the zona incerta, and sham lesions, one week before and three weeks after surgery (indicated by the arrow). Wheel revolutions are expressed as a percent of pre-operative values.


WATER INTAKE (ml)
40
30
20
10


Sham Lesions (n=6)
Dorsal Lesions (n=4)
ZI Lesions (n=8)
WHEEL REVOLUTIONS (%)
250
200
150
100
50
1
2
3
4
TIME (wk)

seven of the eight rats receiving ZI lesions remained depressed throughout the three weeks and it was observed previously (Section 3.1) that the hypodipsia produced by lesions of the ZI does not recover even up to two months after surgery. Finally, lesions dorsal to the ZI also reduced wheel-running to the same extent as did lesions of the ZI, but did not reduce water intake with respect to controls.

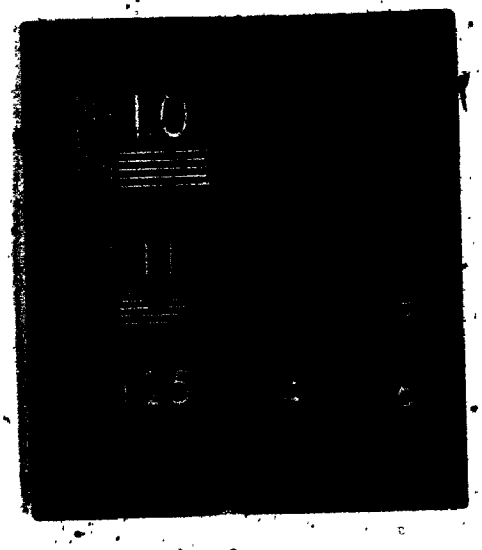
Lesions of the ZI had no significant effect on food intake or rectal temperature (sham-lesioned rats: 37.0 ± 0.1°C; ZI-lesioned rats: 37.1 ± 0.1°C). Changes in body weight gain tended to reflect the changes in activity. In the first week after surgery rats with lesions of the ZI gained slightly more weight than controls ($P < 0.10$), but this was not observed in the second week.

DISCUSSION

The results of the present experiments demonstrate three further characteristics of the reduction in daily water intake after lesions of the ZI. First, the reduced drinking is due to a primary effect on mechanisms controlling water intake and is not secondary to changes in water requirements or losses. Secondly, rats with lesions of the ZI remain in water balance in spite of the reduced fluid intake, by reducing urinary and other water losses. Finally, the effect of the lesions on drinking cannot be attributed to a non-specific depression of behavior.

2 3

OF/DE



The results of the liquid diet experiment and intragastric water-loading experiment indicate that the reduced water intake is not attributable to differences in the post-ingestional use of water by lesioned and control rats. When the fluid intakes of the rats were matched in these two experiments, there were no differences in the volume or concentration of urine excreted, the turnover of electrolytes, or weight changes in the rats. If the reduced intakes after lesioning were due to reduced water requirements or reduced extra-renal water losses, then greater urine volumes should have been excreted by lesioned rats than by controls. Alternatively, if the reduced drinking were due to reduced renal losses and fluid retention, this would have been detected as a lower urine output and in body weight changes. It is concluded that the reduced fluid turnover in rats with lesions of the ZI is caused by a reduction in water intake.

In spite of the reduced water intake, rats with lesions of the ZI appear to remain in water balance. Lesioned animals continue to eat and gain weight normally for long periods after surgery (Section 3.1) and in the present experiment no significant difference was observed between lesioned and control rats in hematocrit or serum osmolality or electrolyte level. Fluid balance was maintained by compensatory reductions in water losses. When water was available ad libitum, the 30% reduction in drinking after lesions of the ZI was accompanied by a 30% reduction

in urine volume. This is in agreement with the observations of Dicker and Nunn (1957) for rats in which water intake was restricted; a 35% reduction in water intake produced a 25% reduction in urine output. In this and the present study, solute loads not significantly different from control levels were excreted due to increases in urine osmolality. The urine osmolalities observed still remain below the maximum concentrating ability of the kidney of the rat (Radford, 1959). Since reductions in urine volume do not account for the entire deficit in water intake (actual volume rather than percentage) either after lesioning or water restriction (Dicker and Nunn, 1957) reductions in extra-renal water losses must also participate in the maintenance of water balance. In addition it should be remembered that rats with lesions of the ZI regulate their water intake as well as controls to intracellular and extracellular water deficits, which would further contribute to the maintenance of body fluid homeostasis.

The last experiment tested the possibility that the reduction in drinking after lesions of the ZI merely reflected a non-specific depression of behavior, since it has been suggested that fibers projecting through the ZI comprise part of the ascending reticular activating system (Shute and Lewis, 1967). There is evidence that arousal level can be an important determinant in the initiation of ingestive activities (Jacobs and Farel, 1971) and recent

studies have suggested that in some cases the effects of brain lesions on feeding and drinking behavior may be related to alterations in arousal levels (Ungerstedt, 1971b). To determine the effect of lesions of the ZI on another spontaneously-occurring behavior in addition to drinking, activity in a running wheel was measured. Although lesions of the ZI reduced wheel-running below control levels, the effect was transient and showed no significant correlation with the reduction of water intake. Lesions dorsal to the ZI produced a similar reduction in this activity but were without effect on ad libitum-drinking. These results suggest that the effects of the lesions on spontaneous activity and drinking are due to damage to close but anatomically distinct neural elements mediating the two. The effect of the lesions on running activity may be attributable to damage to the lemniscal system, the H field of Forel of the extra-pyramidal motor system, or to neural fibers of the ZI involved in general behavioral arousal. Although it is not possible to distinguish between these possibilities from this experiment it is concluded that the reduction in ad libitum water intake after lesions of the ZI is not dependent on a non-specific depression of behavior. A further argument against a general incapacitating effect of the lesions is the observation that daily food intake or drinking in response to water needs is not disrupted by the lesions.

These results demonstrate that lesions of the ZI reduce daily water intake in rats by disrupting neural mechanisms involved in the control of drinking behavior. This is consistent with the observations that chemical (Rindi et al., 1972) and electrical (Huang and Mogenson, 1972) stimulation of the ZI elicit drinking behavior. However, since rats with lesions of the ZI are not deficient in their drinking response to osmotic and volumetric signals of dehydration, it remains to determine the way in which lesions of the ZI affect ad libitum water intake.

3.3 Attenuation of Secondary Drinking by Lesions of the Zona Incerta

The results described in Section 3.1 demonstrate that rats with lesions of the ZI are not deficient in their drinking response to signals of water deficit. As described in the Introduction, however, animals do not drink solely in response to body fluid deficits (primary drinking). Numerous influences not directly related to water needs for fluid balance may initiate and modulate drinking behavior and it has been suggested that it is these secondary factors related to endogenous rhythms, learned associations and environmental influences that actually determine the volume and temporal pattern of water intake (Fitzsimons, 1972). The purpose of the following experiments was to test the hypothesis that lesions of the ZI may reduce ad libitum water intake by attenuating secondary drinking.

The first experiment was designed to determine the effect of lesions of the ZI on drinking occurring in excess of need for body fluid balance. Several authors have reported that rats will continue to drink even when all of their daily water requirements are met through gastric intubations (Fitzsimons, 1971; Kissileff, 1973). In the present experiment, rats were maintained in positive water balance by providing them with a dilute

liquid diet. When additional tap water was made available, the amount of water ingested and the effect of lesions of the ZI on this drinking were determined.

The second experiment was based on the observation of Fitzsimons and LeMagnen (1969) that when daily water requirements were reduced in intact rats, there was an inadequate reduction in water intake due to the persistence of secondary drinking associated with feeding and independent of systemic cues of dehydration. It was hypothesized that if lesions of the ZI attenuate secondary drinking, then lesioned rats should be more dependent on primary signals of water deficit to regulate water intake and should drink more precisely in accordance with actual fluid needs. Daily water requirements were altered in lesioned and control rats by changing diet protein level and the subsequent changesin daily water intake determined.

METHODS

Series 1: Water Intake on a Liquid Diet

This study was carried out in conjunction with that described for Series 2 in Section 3.2 and reports the effect of lesions on additional water intake when rats were maintained on the dilute liquid diet. Tap water and liquid diet (Section 2.1) were available ad libitum. Baseline values were obtained for 24 rats for diet and water intakes and body weight for two weeks and then seventeen of the rats received lesions of the ZI. The remaining seven rats underwent sham-lesioning. Daily intake of tap water and liquid diet and body weight were determined for one week post-operatively. One week later the rats were switched to the solid diet used in the previous experiments and daily food and water intakes determined for six days, to confirm that the hypodipsia observed in the previous experiments was also evident in these animals. At the end of this period the rats were sacrificed, and site and extent of the lesions was determined.

Series 2: Change in Water Intake in Response to a Change in Diet

Nature of the diet is an important determinant of water requirements; a diet rich in protein requires a greater water turnover than one rich in carbohydrate (Radford, 1959). In the present experiment three diets were used, obtained by

altering the protein concentration of the synthetic diet used in the previous experiments (Section 2.1, Table 1). These were: 1) the regular high carbohydrate diet containing 20% casein and 64% sugar by weight (HC diet); 2) a high protein diet in which the amount of casein was increased to 70% at the expense of sugar (HP diet); and 3) an intermediate form in which casein made up 45% of the diet (1/2 HP diet, made from equal amounts of HC and HP diets). The three diets were of approximately equal caloric value. Tap water was provided ad libitum throughout the experiment.

Twenty-four rats were maintained on the regular HC diet for two weeks, during which baseline measures of food and water intake and body weight were taken. Then 16 of the rats were lesioned and eight were randomly selected for sham-lesioning. Water and food intakes and body weight were measured for 41 days following surgery. The regular diet (HC) was replaced by HP diet for six days beginning on the eighth day after surgery and by 1/2 HP diet for ten days beginning on the twenty-fourth post-operative day (see Figure 10). At the end of the experiment the animals were sacrificed and site and extent of the lesions were determined histologically.

RESULTS

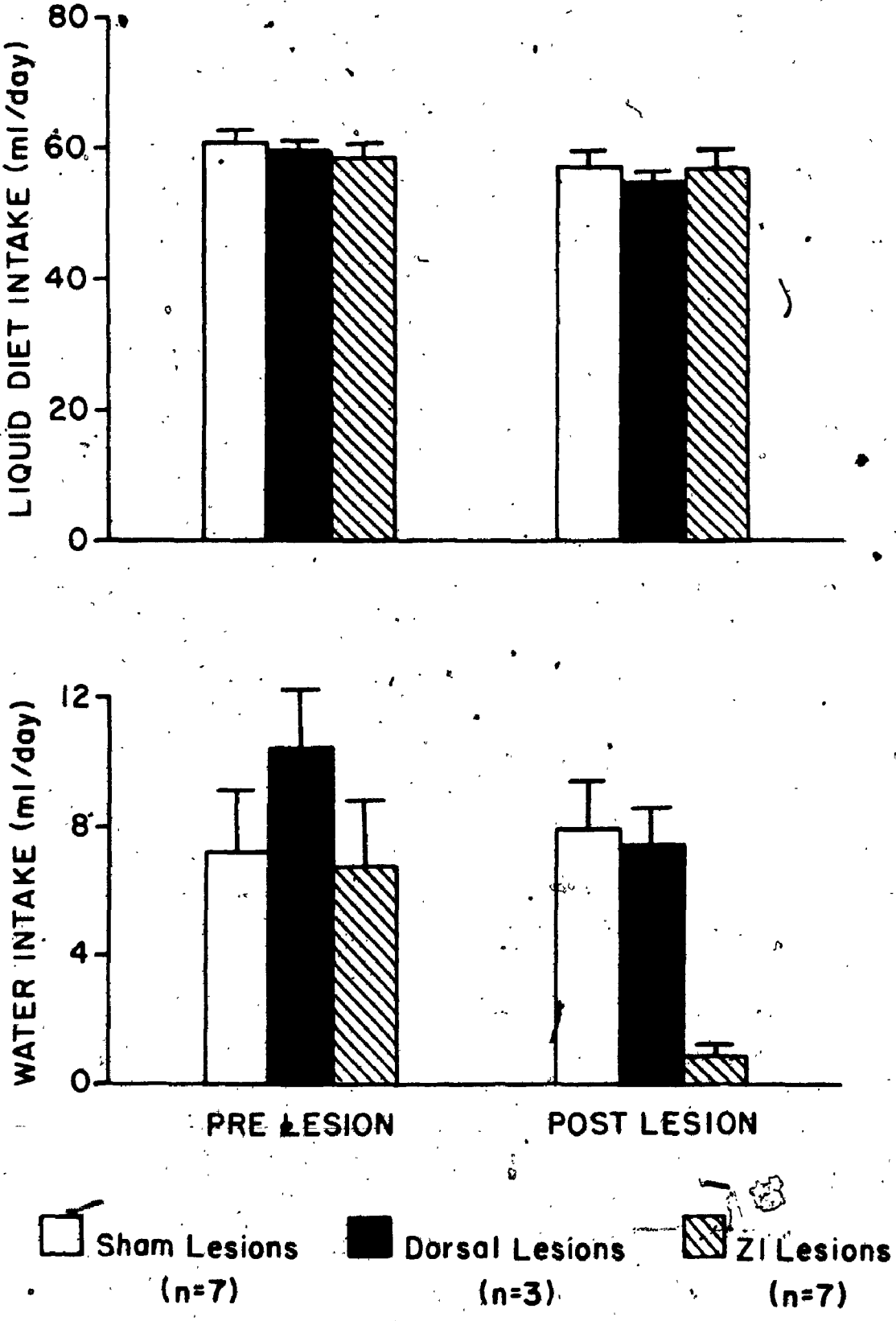
Water Intake on a Liquid Diet

The liquid diet was readily consumed by the rats (Figure 8). A continuous gain in body weight was observed in all rats both before and after surgery, indicating that the diet was adequate to meet nutritional requirements. It was reported in Section 3.2 that when rats were restricted to this diet as the sole source of water, they continued to gain weight normally and excreted large volumes of dilute urine, confirming that the diet was also capable of meeting water requirements for fluid balance. In spite of this, when water was available prior to surgery, the rats drank (Figure 8). This extra drinking was variable at first, increased slowly during the first week and stabilized during the second week.

Lesions of the ZI had no effect on the consumption of liquid diet, but attenuated the drinking of water ($P < 0.01$, Figure 8). In six rats daily water intake fell to less than 1 ml per day, an amount that can probably be accounted for by evaporation and spillage. This attenuation of drinking was not reversed during the week of observation after surgery. Sham lesions produced no change in water intake and control lesions dorsal to the ZI caused only a small decline, perhaps due to slight damage to the ZI (Figure 8). When the animals were placed on the solid diet, the hypodipsia seen in previous experiments in rats with lesions of

FIGURE 8

Intakes of liquid diet and water (mean ± SEM) in rats before and after lesions of the zona incerta (ZI), lesions dorsal to the zona incerta, and sham lesions.


LIQUID DIET INTAKE (ml/day)
80
60
40
20
0
WATER INTAKE (ml/day)
12
8
4
0
PRE LESION
POST LESION
Sham Lesions
(n=7)
Dorsal Lesions
(n=3)
ZI Lesions
(n=7)

the ZI was observed; rats with lesions of the ZI drank less than sham-lesioned or control-lesioned rats, but consumed similar amounts of food (Figure 9).

Change in Water Intake in Response to a Change in Diet

Daily food and water intakes for rats receiving lesions of the ZI and for sham-lesioned control rats are presented in Figure 10. As observed in the previous experiments, lesions of the ZI produced a significant reduction in ad libitum water intake ($P < 0.01$) but not in food intake.

The changes in daily water intake accompanying the changes in diet composition were very different for the two groups (Figure 10). The results for the sham-operated control rats confirm and extend the observations of Fitzsimons and LeMagnen (1969); changes in water intake were not as predicted solely on the basis of expected changes in water requirements. Water intake rapidly increased when diet protein was increased, but, except for an initial drop, failed to return to previous levels when HC diet was returned. The increase in intake induced by the second diet change to 1/2 HP diet was less than 1/3 that induced by the change to HP diet. The results for rats with control lesions dorsal to the ZI ($N = 3$) were not significantly different from those of the sham-operated controls.

Water intake in rats with lesions of the ZI, however, varied in a regular manner with alteration in diet protein

FIGURE 9

Intakes of solid food (see text, Section 3.3) and water (mean ± SEM) in rats after lesions of the zona incerta (ZI), lesions dorsal to the zona incerta, and sham lesions.

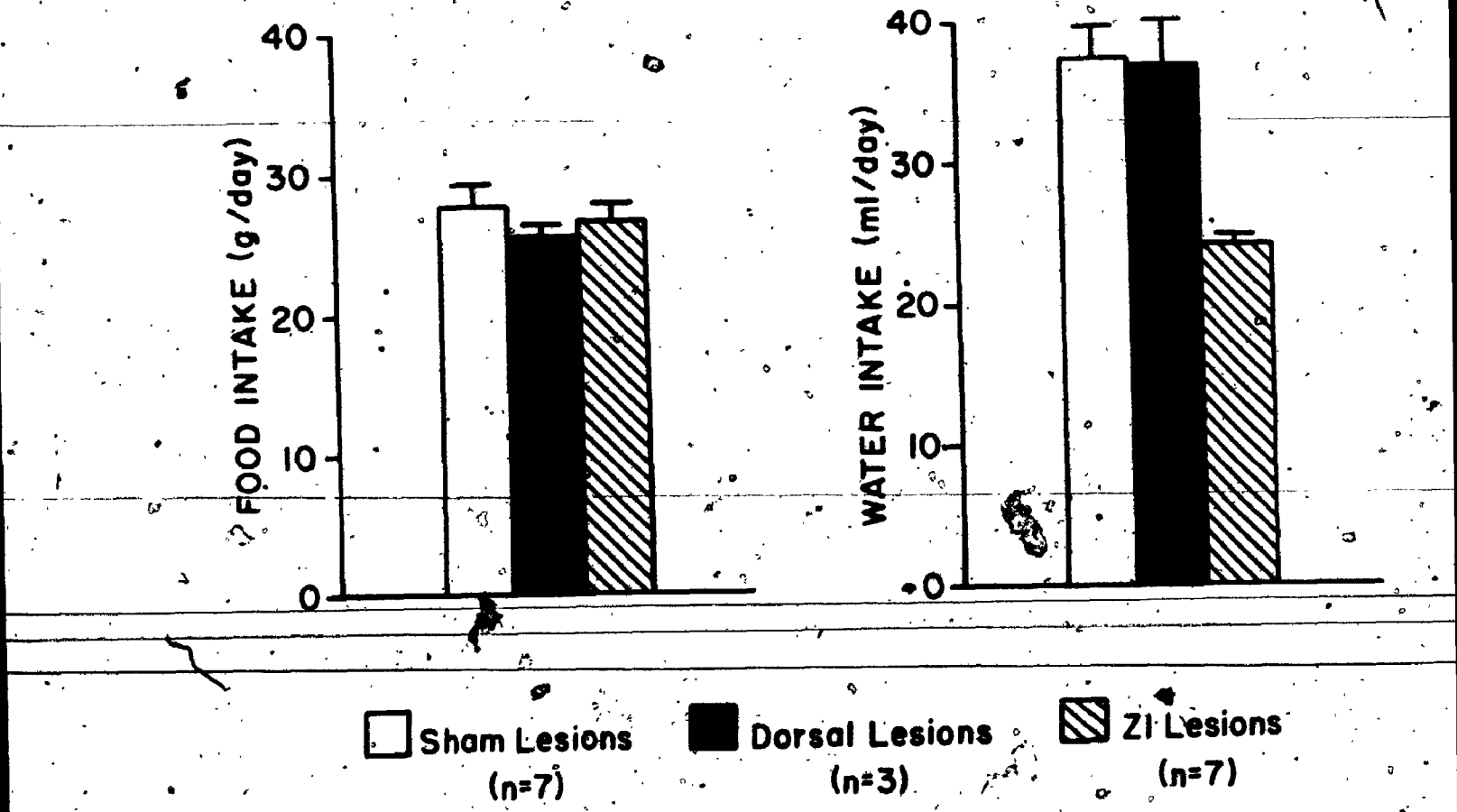
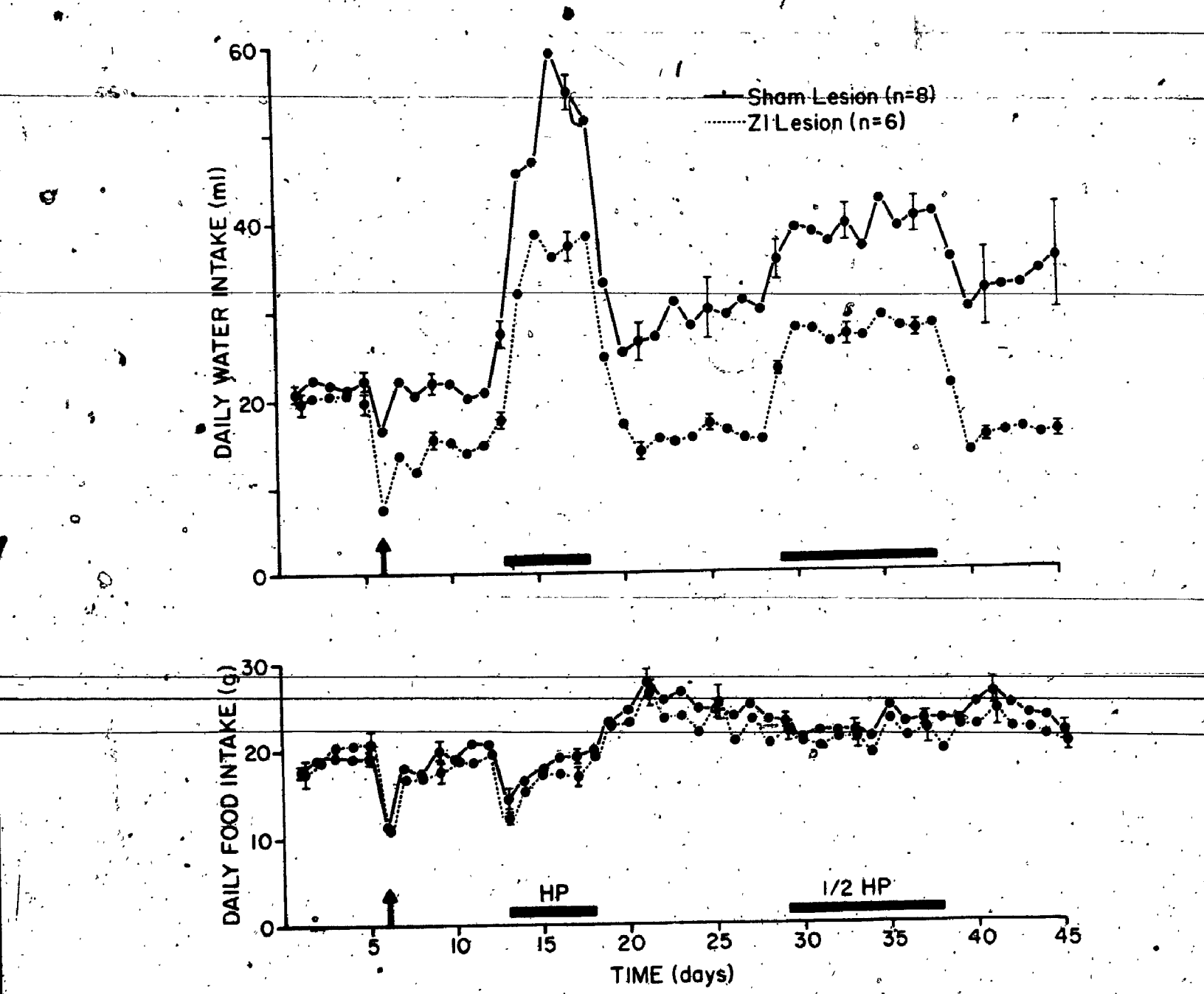

40
30
20
10
0
FOOD INTAKE (g/day)
40
30
20
10
0
WATER INTAKE (ml/day)
Sham Lesions
(n=7)
Dorsal Lesions
(n=3)
ZI Lesions
(n=7)

FIGURE 10

Effect of changes in diet protein content on daily water and food intakes of rats receiving lesions of the zona incerta (ZI) and sham-lesioned controls. Data points are means; SEM is presented for every fourth day. Surgery was performed on Day 6 (arrow) and the solid horizontal bars represent periods when the protein content of the diet was increased (see text, Section 3.3).


Sham Lesion (n=8)
ZI Lesion (n=6)
DAILY WATER INTAKE (ml)
60
40
20
0
DAILY FOOD INTAKE (g)
30
20
10
0
HP
1/2 HP
5
10
15
20
25
30
35
40
45
TIME (days)

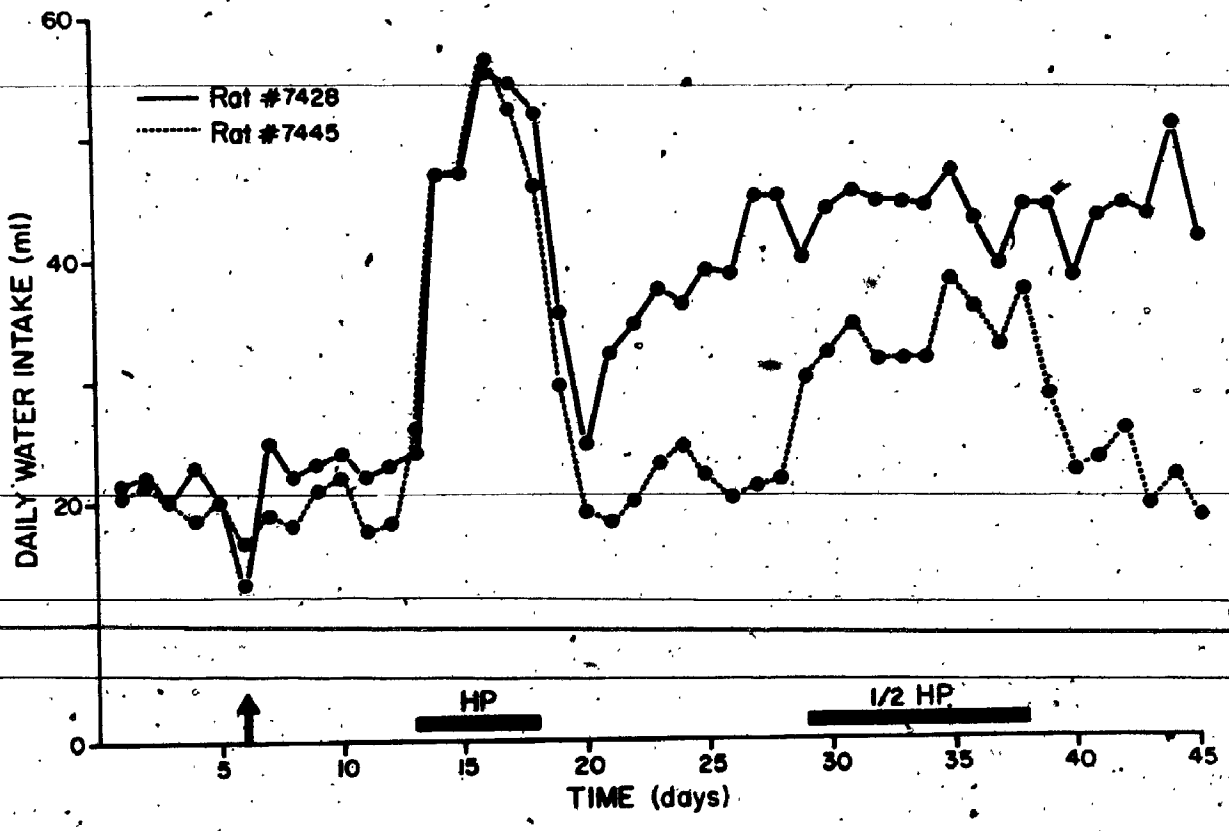
level. Drinking increased when diet protein level was increased and promptly returned to lower levels when diet protein content was reduced. Furthermore, the increase in water intake induced by the 1/2 HP diet was approximately one-half that induced by the HP diet.

As reported in the initial experiments, daily water intake was less variable between lesioned rats than controls and this was most evident at the end of the experiment (Figure 10). Within the sham-lesioned group there was much variability in the response to the diet changes; the two extremes are presented in Figure 11. Rat 7445 behaved more like rats with lesions of the ZI, although water intake was higher. Rat 7428, however, continued to ingest large quantities of water after the first diet change and except for the second day after transition from HP diet back to HC diet daily water intake remained elevated and continued to increase slightly. No further increase was observed when the second diet change was initiated, suggesting that this rat was already consuming enough water to meet the increased demand.

Differences in food intake cannot account for the differences in water intake (Figure 10). There was no significant difference in ad libitum food intake between rats with lesions of the ZI, lesioned controls, or sham-lesioned controls. There was also no significant difference in body weight gain.

FIGURE 11

Effect of changes in diet protein content on daily water intake of two rats receiving sham lesions. Surgery was performed on Day 6 (arrow) and the solid bars represent periods when the protein content of the diet was increased (see text, Section 3.3).


60
40
20
0
DAILY WATER INTAKE (ml)
Rat #7428
Rat #7445
HP
1/2 HP
5
10
15
20
25
30
35
40
45
TIME (days)

DISCUSSION

The results of the experiments provide further evidence that drinking in rats under ad libitum conditions is not restricted to actual water requirements for fluid balance and suggest that lesions of the ZI reduce ad libitum water intake by attenuating this extra drinking.

Fitzsimons (1971) observed that the continuous intragastric infusion of water in volume sufficient to maintain water balance only slightly suppressed ad libitum drinking. The results of the liquid diet experiment confirm that intact rats will continue to ingest water when positive water balance is maintained. Rats restricted to this liquid diet continue to gain weight normally and excrete large volumes of dilute urine (Section 3.2), attesting to its effectiveness in meeting fluid requirements. An additional feature of using the liquid diet is that fluid replacement occurs concomitantly with feeding, since rats normally consume most of their daily water in close temporal association with meals (Kissileff, 1969a). However, when water was available, drinking still occurred. The ingestion of extra water did not reduce the intake of liquid diet so that total fluid intake rose even higher. Lesions of the ZI attenuated this excess drinking, but did not reduce the intake of liquid diet.

The response of the control rats to the changes in daily water requirements confirms and extends the observa-

tions of Fitzsimons and LeMagnen (1969), providing further evidence that under ad libitum conditions drinking is not strictly dependent on actual water needs for fluid balance. When water requirements were increased, by increasing diet protein levels, daily water intake increased; however, reductions in water intake did not strictly follow reductions in water need when diet protein level was reduced. As in the study by Fitzsimons and LeMagnen, drinking decreased slowly and less completely than expected. Furthermore, a second increase in water requirement failed to increase intake as much as expected, presumably because excess water being consumed after the first change could partially offset the new increase in need. This was especially obvious in rat 7428 (Figure 11) that continued to consume enough water after the first diet change that no further increase was observed when water requirements were again increased. If control rats were responding solely to systemic cues for the regulation of drinking, then water intake should have varied more precisely with changes in water requirements. Instead, it has been shown (Fitzsimons and LeMagnen, 1969) that learned associations between feeding pattern and water requirements are established and at least partially determine total fluid intake. Even when water requirements are reduced, these learned associations persist.

The water intake of rats with lesions of the ZI, however, did follow quite closely the expected changes in water

requirements. This confirms that lesioned rats respond to their water needs for fluid homeostasis but indicates that unlike control rats they regulate their drinking more precisely to these needs. This is consistent with observations in this and the previous experiments that lesions of the ZI tended to reduce water intake to a rather constant level regardless of pre-lesion intake and that daily water intake was less variable between lesioned rats than controls. These results suggest that lesions of the ZI reduce ad libitum water intake by attenuating secondary drinking.

The reduction in drinking after lesions of the ZI appears to be of little consequence to the rats, at least in the laboratory environment. Through compensatory reductions in urine and other body water losses, water balance is maintained and lesioned rats continue to eat and gain weight normally (Section 3.2). This does not imply, however, that secondary drinking is insignificant in the control of normal ad libitum drinking. Nor do the observations that lesions of the ZI reduce daily water intake 25%, or that drinking in normal rats can be restricted by a similar amount without compromising fluid homeostasis (Dicker and Nunn, 1957), imply that this is the fraction of ad libitum intake that is secondary. In fact, it has been suggested that all of an animal's ad libitum intake is controlled by secondary influences (Fitzsimons, 1972). In the following section the effect of lesions of the ZI on the daily pattern of ingestion is examined.

Finally, it still remains to determine the way in which lesions of the ZI reduce secondary drinking. The results of the present experiments are consistent with a hypothesis that neural elements of the ZI are specifically involved in the mediation of drinking responses to secondary cues. However, several experimental manipulations which make drinking more difficult (bar pressing for water: Morrison, 1968) or less attractive (unpalatable solutions: Pfaffmann, 1960) also reduce ad libitum water intake. It is important to consider that lesions of the ZI may act in an analogous manner through subtle changes in the ability to drink or a general inhibition of water intake. This problem is examined in Section 3.5.

3.4 Daily Patterns of Ingestion after Lesions of the Zona Incerta

Daily patterns of ingestion in the rat have been described by several authors (Fitzsimons and LeMagnen, 1969; Kissileff, 1969a) and under stable conditions are regular from day to day; the most obvious features are a circadian rhythm and a close temporal relationship between feeding and drinking. There is evidence to suggest that these patterns may be controlled at least in part by secondary influences such as endogenous rhythms and learned associations (Fitzsimons, 1972; Oatley, 1973). Since the previous experiments suggest that lesions of the ZI disrupt secondary drinking but leave responses to water deficits intact, it is relevant to consider the effects of lesions of the ZI on spontaneous patterns of water intake.

Food jars and water spouts were equipped with devices to record automatically feeding and drinking behavior throughout the day. Besides characterizing the daily pattern of intake in rats after lesions of the ZI, it was also of interest to determine the immediate effect of lesioning. It was hypothesized that if the factors which normally regulate water intake are secondary cues and if lesions of the ZI attenuate secondary drinking, then the initial effect of lesions of the ZI should be a temporary attenuation of drinking until initiated by signals of dehydration. In the

previous experiments immediate lesion effects have been complicated by recovery from anesthesia. It has been shown, however, that electrolytic lesions can be made in awake animals with little or no trauma by passing current between the tips of implanted bipolar electrodes (Peters and Sensig, 1974).

METHODS

Recording Apparatus

The occurrence of drinking was recorded by using a drinkometer circuit initially described by Hill and Stellar (1951) and obtained commercially from Grason-Stadler Co., Mass. Briefly, one lead from a relay switch was positioned in the drinking tube in contact with the water and the other was attached to the bottom of the cage. As the rat lapped the water in the spout it completed the circuit to the relay. The current passed was less than 0.1 μa.

An identical circuit was used to monitor feeding. Food jars were equipped with screw-cap lids with a hole cut in the center 3 cm in diameter. A piece of glass tubing was bent to pass up the outside of the food jar and insert into a small hole cut into one side of the jar lid. This was cemented with acrylic to the lid. A flexible insulated wire was passed through the glass tubing and on the underside of the jar lid soldered to a circular piece of wire screen (8 mm mesh) of diameter slightly larger than that of the central hole in the lid. When the lid was placed on the

food jar the screen rested on the surface of the food. The rat would eat through the screen and as he consumed the food would push the screen down further. Preliminary tests with this device were carried out to ensure that the rats could eat freely and could easily obtain more than 40 g of food from the jar. After this the jars were filled daily. To record feeding, the wire from the food jar and a contact to the bottom of the cage were connected to a drinkometer circuit as described above.

The water spout and food jar were mounted in a cage identical to that in which the rats were regularly housed. The Richter tube water spout (identical to those used previously) was inserted into the cage. To ensure the recording of discrete laps, a small metal guard was mounted over the spout. The hole in the drinking spout was positioned 2 mm below a hole 1 cm in diameter in the guard. The purpose of the guard was to prevent the rat from making continuous contact with the spout with its paw or mouth. Preliminary experiments with this apparatus indicated that no reduction in daily water intake occurred when rats were drinking from these spouts, either with the current turned on or off.

Four cages were prepared with the feeding and drinking devices. Connecting cables from the cages were passed outside the room in which the rats were housed to cumulative recorders (Ralph Gerbrands Co., Mass.). As illustrated in

Figure 12 laps at the drinking spout were recorded by upward excursions of the pen and contact with the feeding device as short downward deflections. Paper speed was set at 30 cm/hr.

Procedure

Twenty-two rats were individually housed and maintained in a room lighted from 6 AM to 6 PM. The regular granular diet (HC diet) and water were available ad libitum. One week later bipolar electrodes (Plastic Products Co., Roanoke, Va., insulation scraped 1/3 mm from tips and tips separated by 1/3 mm) were implanted bilaterally in the region of the ZI and cemented in place with dental acrylic. The rats were allowed three weeks to recover. During the last week of this period the food jars were equipped with lids and screens identical to those described above, to adapt the rats to the feeding devices.

The 22 rats were run through the following sequence, 4 at a time. Animals were placed in the recording cages and spontaneous patterns of food and water intake were recorded for at least 5 days. Lesions were then made through the implanted electrodes (see below) and intakes were recorded for the next 5 days. Two weeks later the rats were returned to the recording apparatus for 4 consecutive days. The animals were then sacrificed and site and extent of the lesions determined histologically.

Lesions were made by passing 1 ma of direct current between the tips of the implanted bipolar electrodes for 5 sec. Eight rats received lesions at 5 PM (1 hr before dark) and were returned to the recording cages immediately. To reduce the initial burst of feeding and drinking that was observed in these rats (see Figure 13 and Results section), the remaining 14 rats received lesions at 4 PM and were placed in empty cages for 1 hr. All rats were sham lesioned (connected to the lesioning device, then returned either immediately or 1 hr later) on the day before receiving the actual lesions.

Data Analysis

As reported by others (Fitzsimons and LeMagnen, 1969; Kissileff, 1969a), feeding occurred in discrete meals. Based on Kissileff's (1970) observations on meal frequency and duration, a meal was defined as a bout of eating not interrupted by more than 10 min. Drinking also tended to occur in discrete bursts and a drink was defined as laps of water not separated by more than 5 min or by feeding. Drinks sometimes consisted of two or more continuous drafts of water with short periods in between (visual observation indicated that these interruptions were frequently due to brief periods of grooming). Drafts of water were defined as periods of lapping not separated by more than 1 min or by feeding (Kissileff, 1970). Drink or draft size was determined by multiplying the number of laps recorded by a factor

obtained by dividing the total volume of water ingested in the day by the total number of laps recorded. To quantify the relationship between feeding and drinking four periods were defined: 1) 10 minutes before a meal, 2) during a meal, 3) 10 minutes after a meal, and 4) between meals. Water intake during each of these periods was determined and expressed as a percent of total daily intake (Figure 14). The probability of drinking during any of these periods was calculated as the fraction of the periods in which drinking occurred (Figure 15).

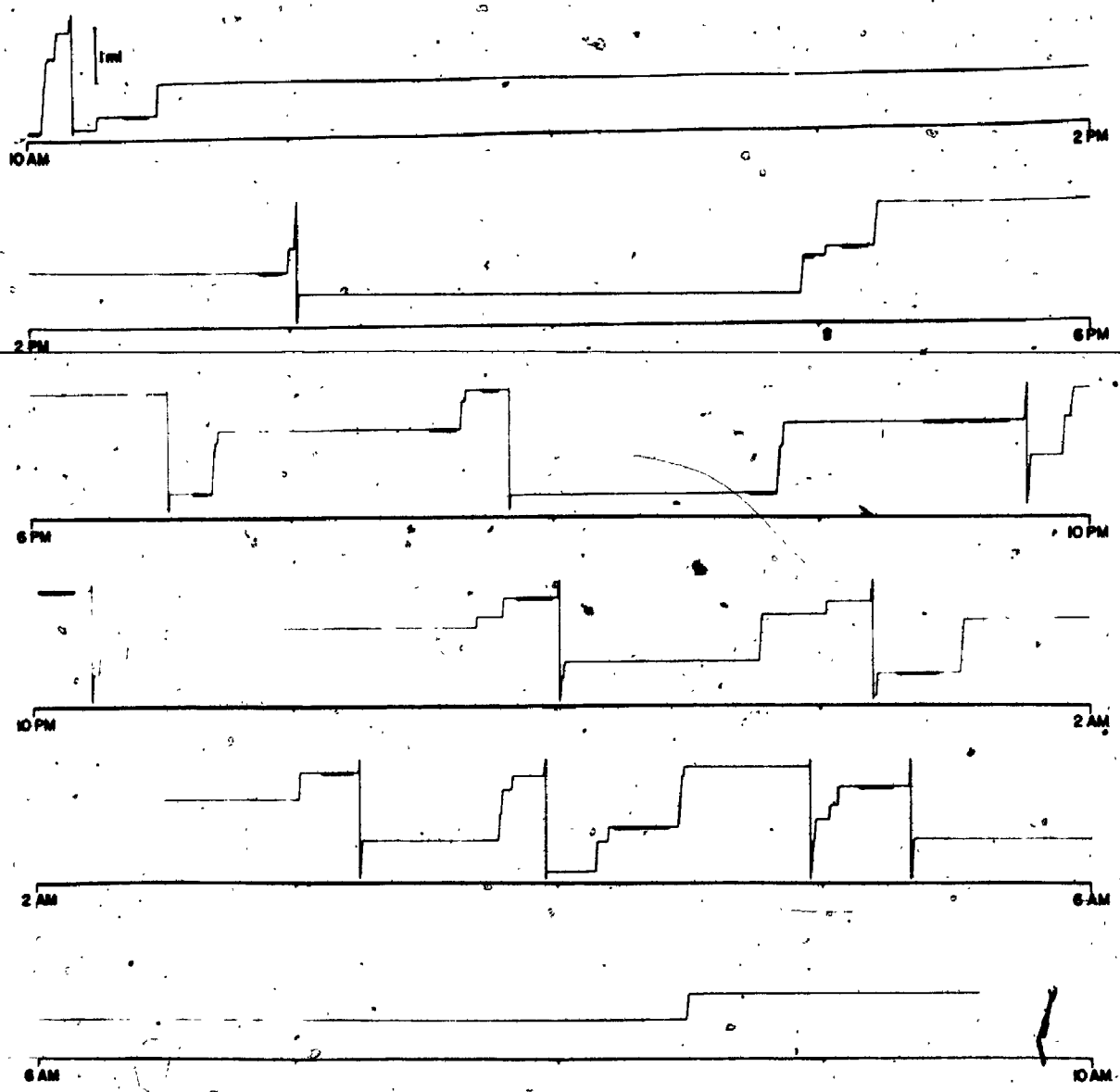
RESULTS

Patterns of Ingestion before Lesioning

An example of the continuous records of feeding and drinking obtained before lesioning is presented in Figure 13. Quantitative analysis of these records was based on averages for each rat of the two days immediately before lesioning; the results are presented in Table 5 and Figures 14 and 15. The patterns were similar to those observed by others (Fitzsimons and LeMagnen, 1969; Nissileff, 1969a, 1970). Feeding and drinking occurred in discrete bouts throughout the day, but a definite circadian rhythm was evident; 70-80% of the meals and daily water intake were ingested at night. A close temporal association between feeding and drinking was also observed; 76% of total water intake occurred within 10 minutes before or after a meal (Figure 14) and 76% of the

FIGURE 12

Continuous record illustrating the temporal pattern of feeding and drinking in one rat for the day before lesioning. Tongue contacts with the drinking spout were recorded as upward excursions of the pen (resets at the top of the record) and contact with the feeding device as short downward deflections (thick horizontal portions of line, due to close vertical displacements, constitute a meal). The lower trace records time; lights were off from 6 PM to 6 AM. At about 5 PM (indicated by arrow) this rat was sham-lesioned (see text, section 3.4) and returned immediately to the recording cage.


1 ml
10 AM
2 PM
2 PM
6 PM
6 PM
10 PM
10 PM
2 AM
2 AM
6 AM
6 AM
10 AM

meals were followed by drinks (Figure 15).

Lesion Site

In this experiment 15 rats sustained lesions of the ZI but were separated into two categories: one group (N = 7) sustained lesions centered on the ZI with little or no extension ventrally into the lateral hypothalamus (LH) and is referred to as the ZI (dorsal) lesioned group; the second group (N = 8) received lesions of the ZI with partial extension into the LH (less than 0.5 mm) and is referred to as the ZI (ventral) group. These two groups were treated separately for data analysis. Six rats sustained lesions dorsal to the ZI and constituted the control lesioned group.

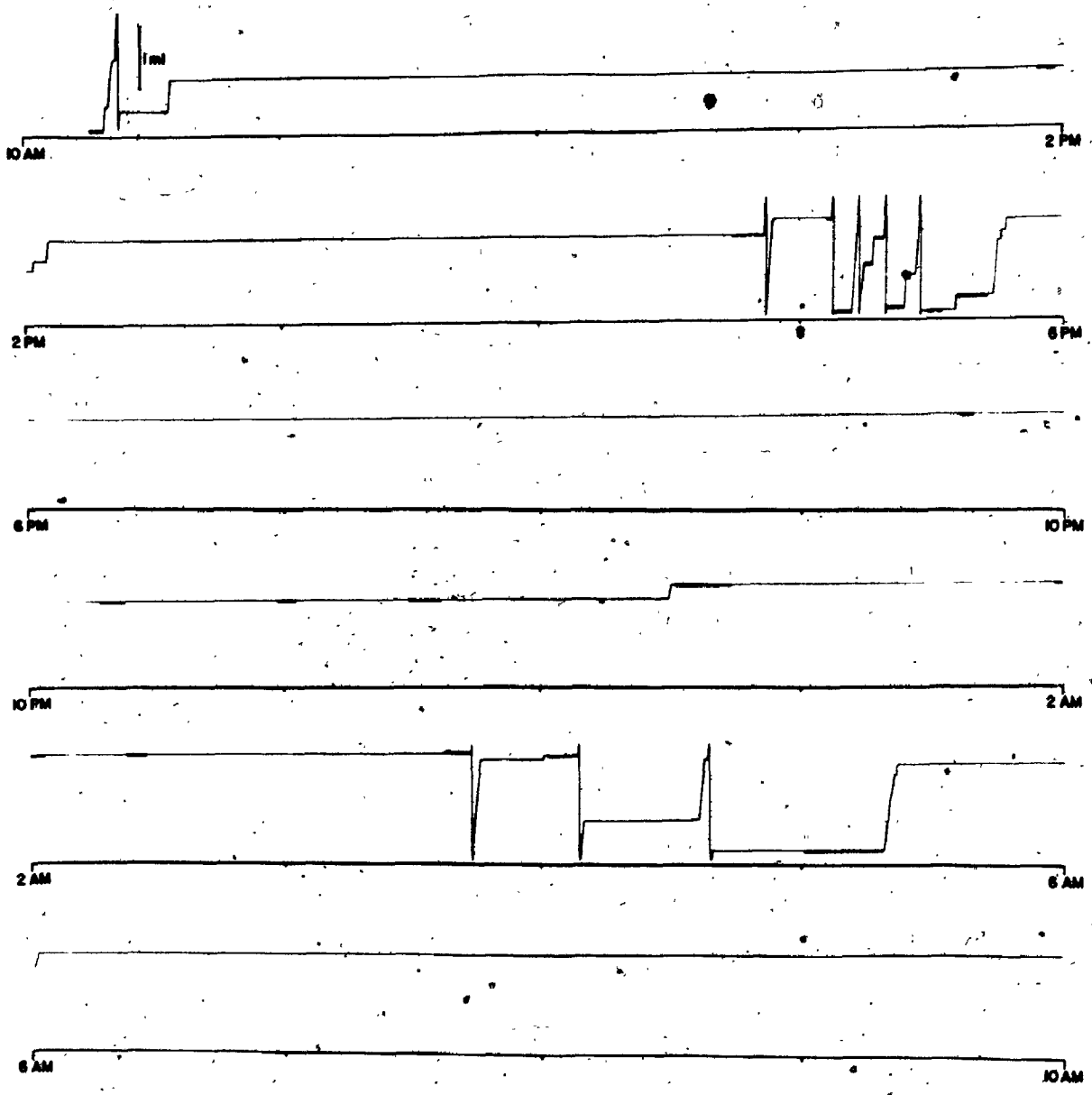
Immediate Lesion Effects

The lesioning procedure caused only minor disturbance in the rats. Typically, their bodies tensed for 2 or 3 seconds while the current was passed and then they relaxed.

The record of feeding and drinking for a rat that was lesioned at 5 PM and returned to the cage immediately is presented in Figure 13; histological examination indicated that the lesions were placed in the ZI with no extension into the LH. After a short period of grooming, this rat engaged in a burst of alternate feeding and drinking (Figure 13) quite different from the single meal and drink observed after sham lesions (Figure 12). This burst of activity was observed in 4 of the 8 rats returned to the recording cages

FIGURE 13

Daily record of feeding and drinking (as in Figure 12) in a rat receiving lesions of the zona incerta at 5 PM (indicated by the arrow) through implanted electrodes and returned immediately to the recording apparatus.


1 ml
10 AM
2 PM
2 PM
6 PM
6 PM
10 PM
10 PM
2 AM
2 AM
6 AM
6 AM
10 AM

immediately after lesioning and lasted about 1/2 hr with the ingestion of 4.5-7 ml of water in 6-9 drinks. These 4 rats received ZI (dorsal) lesions. Of the remaining 4 rats, 2 exhibited a burst of feeding similar to that described above but ingested only 1 ml of water in 5 drinks, while the other 2 engaged in periodic nibbling for 3/4 hr with only a few laps at the water spout. These 4 rats received ZI (ventral) lesions.

Fourteen rats were placed in empty cages for 1 hr after lesioning before being returned to the recording apparatus. During this time they engaged in quiet exploration, grooming and sleeping; there was no sign of hyperactivity. When rats with ZI (dorsal) lesions (N = 3) were returned to the recording cages 1 hr later the initial burst of feeding and drinking was reduced. A bout of eating similar to that described above was observed, but was interrupted by fewer drinks (1-4) and less water was ingested (0.4-3.9 ml). Rats with lesions dorsal to the ZI (N = 5) also ate but drank more water (4.1-6.5 ml in 2-4 drinks). In rats with lesions extending into the LH (N = 4) a brief period (15-30 min) of nibbling was observed with few laps at the spout.

Following this initial period all lesioned rats exhibited a transient depression of eating and drinking. In rats with lesions restricted to the ZI eating resumed between 8 and 10 PM, occurring in the normal pattern of discrete meals (see Figure 13). Unlike the normal pattern, however,

no drinking accompanied this feeding and 2-4 meals occurred before drinking resumed. Lesions dorsal to the ZI, which may have partially damaged neural elements of the ZI, also produced a transient depression of feeding and drinking but eating resumed slightly earlier than in lesioned rats (6:30-8 PM) and drinking occurred either with the first or second meal. In one rat receiving asymmetric lesions which spared much of the medial ZI eating and drinking resumed at 6:30 PM.

Rats with lesions extending into the LH showed the greatest depression of eating and drinking; over half of these rats ingested less than 5 g of food or 5 ml of water on the day of lesioning, and all but one ingested less than 10 g of food or 10 ml of water. In most of these animals nibbling rather than discrete meals was observed. In the morning after lesioning these rats were tested with an open water dish for 15 min but did not drink. The hypophagia and hypodipsia persisted for a second day but daily food intake recovered to control levels by the third or fourth day. All were eating in discrete meals by the third day.

Patterns of Ingestion after Lesioning

Following the initial disruption after lesioning, feeding and drinking patterns quickly stabilized. Quantitative analysis of these patterns was carried out and the results are presented for the average of the last 2 days of the recording period 2 1/2 weeks after lesioning (Table 5, Figures 14 and 15).

TABLE 5. CHARACTERISTICS OF FEEDING AND DRINKING PATTERNS BEFORE AND AFTER LESIONING

Group	Food Intake (g/day)	Number of Meals	Water Intake (ml/day)	Number of Drinks	Mean Drink Size (ml)	Number of Laps per day	Volume Ingested per Lap (µl)
Before Lesioning (N = 22)	22.3±0.6	12±1	22.7±0.8	21±1	1.15±.06	4510±240	5.2±0.9
Control Lesions (N = 6)	20.3±1.1	14±2	20.7±0.6	19±1	1.21±0.5	3660±300	5.8±0.4
ZI (dorsal) Lesions (N = 7)	18.6±0.7	12±1	17.2±0.8†	26±4*	0.74±.11†	3750±330	4.8±0.4
ZI (ventral) Lesions (N = 8)	20.3±0.8	18±2	17.1±0.9†	25±3*	0.70±.06†	4160±430	4.7±0.5

Values are means ± SEM calculated from the average of 2 days for each rat (see text, Section 3.4).

* significantly different from the value for the control lesion group, $P < 0.05$.

† significantly different from the value for the control lesion group, $P < 0.01$.

There was no significant difference in daily food intake between the three groups (Table 5, $P > 0.20$). Rats with ZI (ventral) lesions, however, ate more frequently and in smaller meals (Table 5), and those rats with the greatest extension of the lesions into the LH showed the largest increase in meal frequency. There was no change in the pattern of eating in rats with ZI (dorsal) lesions. The rate of eating within meals was calculated by dividing the total amount of food ingested in the two days by the total time spent eating. This varied between 0.17 and 0.34 g/min and did not differ significantly between groups ($P > 0.20$).

As observed in the previous experiments, lesions of the ZI reduced ad libitum water intake (Table 5, $P < 0.01$). This hypodipsia was characterized by a smaller mean drink size ($P < 0.01$) and a slight increase in drink frequency ($P < 0.05$). There was a significant correlation between mean drink size and daily water intake after lesioning ($N = 22$, $r = 0.49$, $P < 0.05$). The distribution of drink and draft size for rats in each of the 3 lesion groups is presented in Figure 16, and illustrates the shift towards the ingestion of smaller volumes of water in rats with lesions of the ZI; in all groups the longer drinks tended to occur immediately after eating. The smaller mean drink size and reduction in total daily water intake, however, were not due to a reduced number of laps at the spout (Table 5). Thus rats with lesions of the ZI were engaging in as much drinking as

FIGURE 14

Temporal relationship between ad libitum feeding and drinking behavior in rats before and after lesioning. The percent of daily water intake occurring within 10 minutes before, during, or 10 minutes after meals or between meals is presented in the top panel (before lesioning). Changes in percent intake during these periods after lesioning are presented in the bottom panels. Bars are means ± SEM, calculated from the average of 2 days for each rat.

* significantly greater than zero, $P < 0.05$

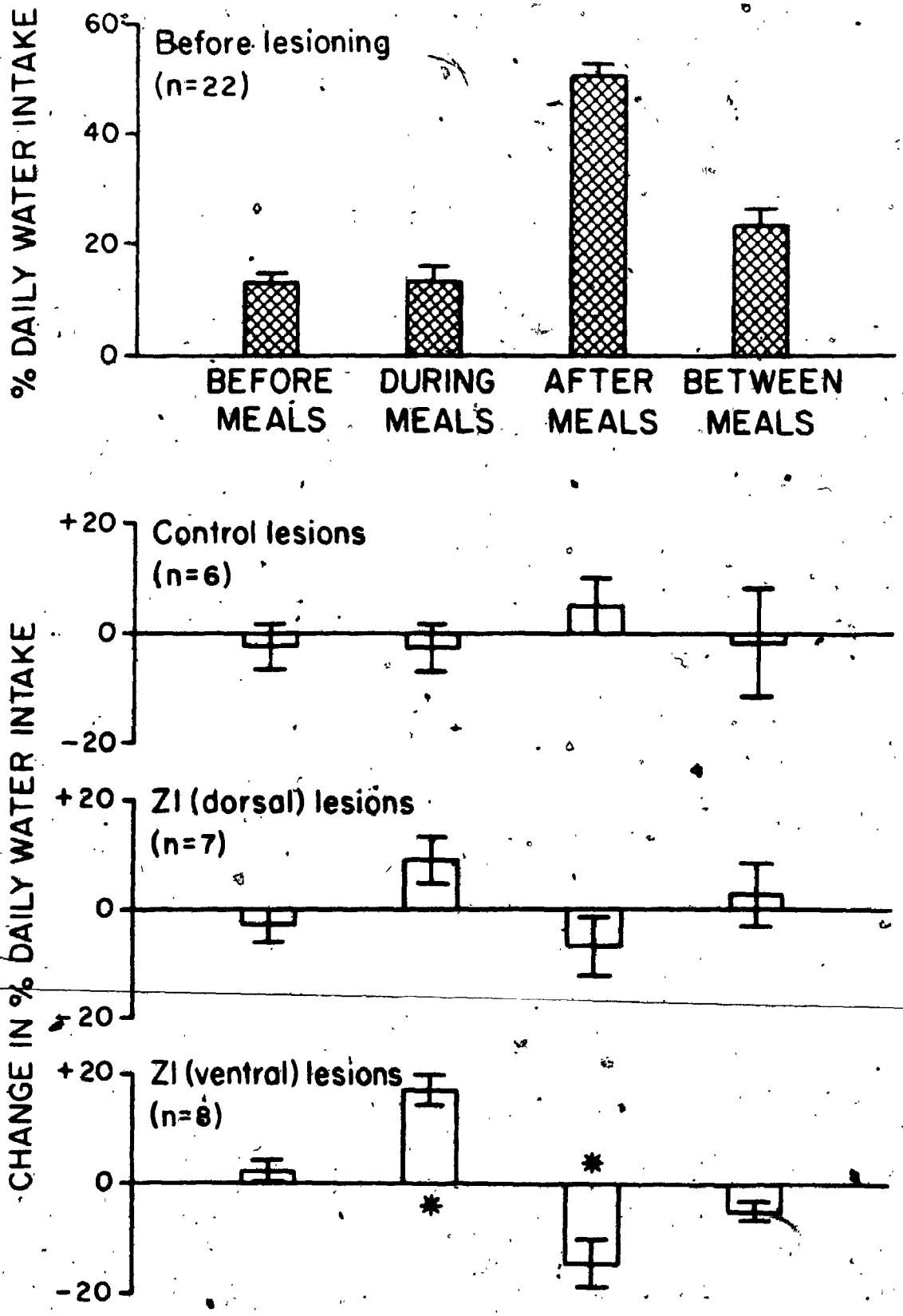

% DAILY WATER INTAKE
60
40
20
0
Before lesioning
(n=22)
BEFORE MEALS
DURING MEALS
AFTER MEALS
BETWEEN MEALS
CHANGE IN % DAILY WATER INTAKE
+20
0
-20
Control lesions
(n=6)
ZI (dorsal) lesions
(n=7)
ZI (ventral) lesions
(n=8)
*
*

FIGURE 15

Temporal relationship between ad libitum feeding and drinking behavior in rats before and after lesioning. The probability of drinking occurring within 10 minutes before, during, or 10 minutes after a meal or between meals is presented in the top panel. Changes in these probabilities after lesioning are presented in the bottom panel. Bars are means ± SEM, calculated from the average of 2 days for each rat.

* significantly greater than zero, $P < 0.05$

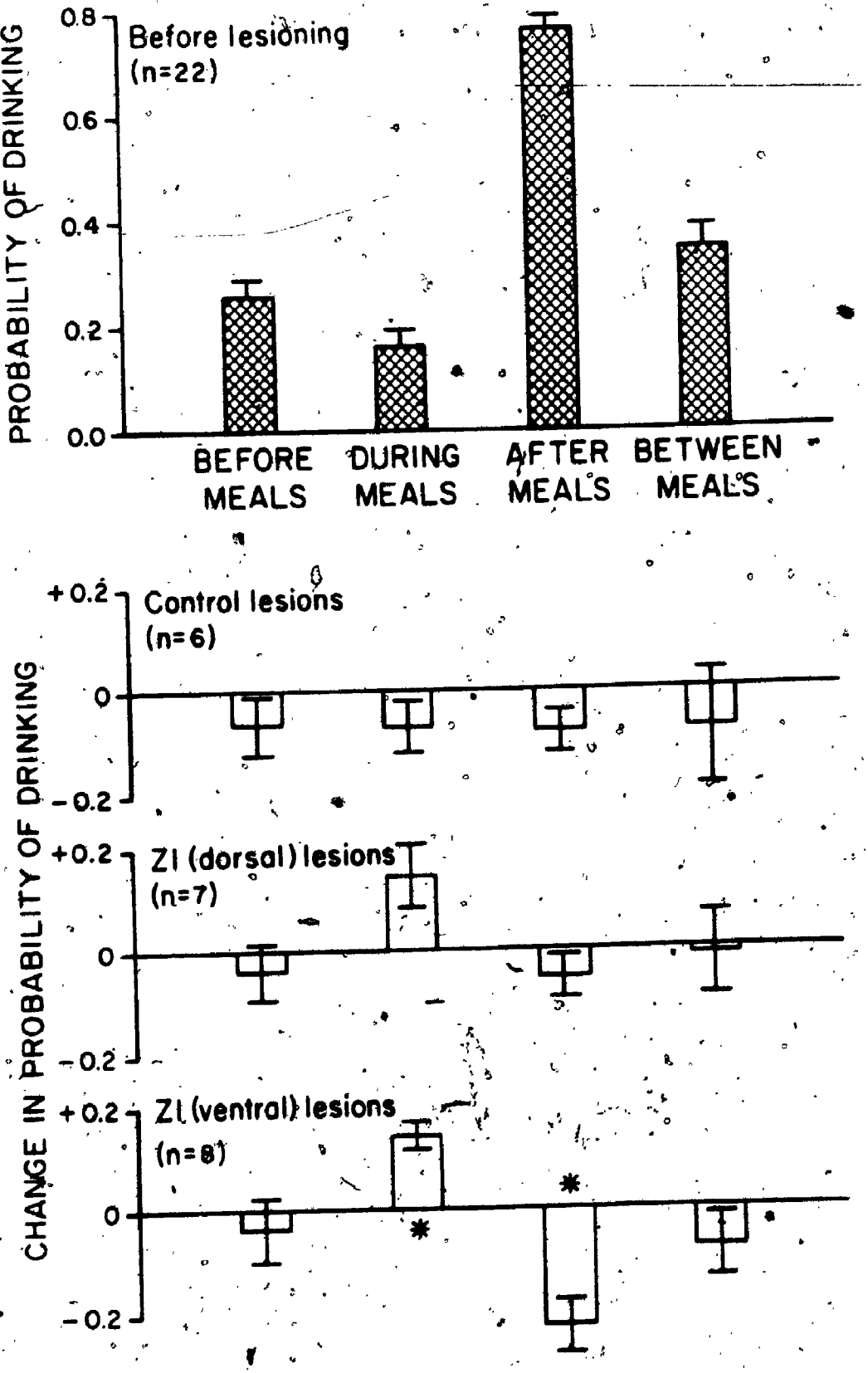
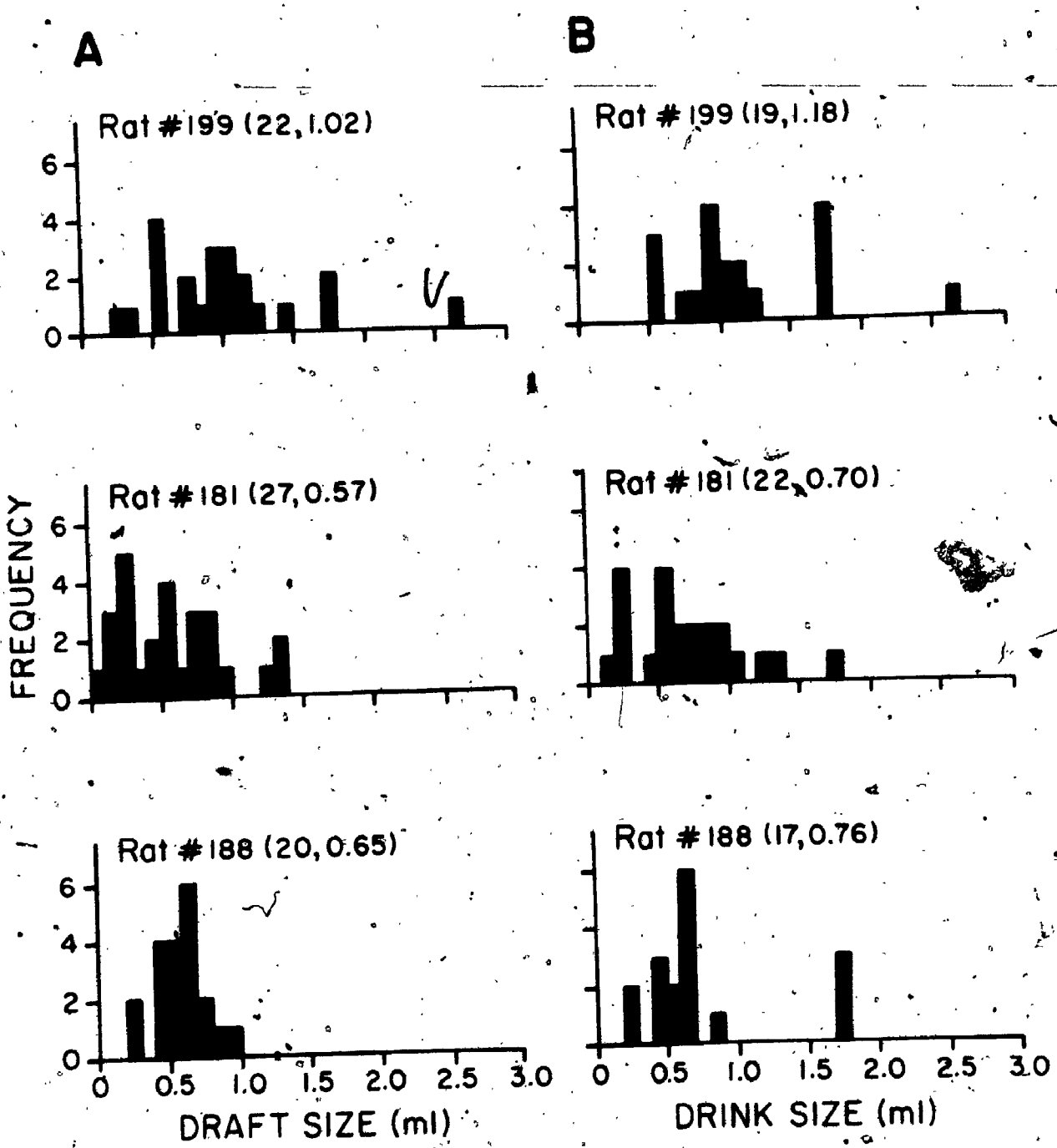

PROBABILITY OF DRINKING
0.8
0.6
0.4
0.2
0.0
Before lesioning
(n=22)
BEFORE MEALS
DURING MEALS
AFTER MEALS
BETWEEN MEALS
CHANGE IN PROBABILITY OF DRINKING
+0.2
0
-0.2
Control lesions
(n=6)
ZI (dorsal) lesions
(n=7)
ZI (ventral) lesions
(n=8)

FIGURE 16

Size distribution of drafts (A) and drinks (B) ingested over 24 hours in three rats. Rat 199 received control lesions dorsal to the zona incerta, rat 181 received lesions of the zona incerta, and rat 188 received lesions of the zona incerta which partially extended into the lateral hypothalamus. Values in parentheses are total number of drafts or drinks and mean draft or drink size for the 24 hour period. (For definition of drinks and drafts, see page 111.)


A
B
Rat #199 (22, 1.02)
Rat # 199 (19, 1.18)
Rat # 181 (27, 0.57)
Rat # 181 (22, 0.70)
Rat # 188 (20, 0.65)
Rat # 188 (17, 0.76)
FREQUENCY
0
2
4
6
0 0.5 1.0 1.5 2.0 2.5 3.0
DRAFT SIZE (ml)
DRINK SIZE (ml)

controls but were obtaining less water; this is shown in Table 5 as a reduction in the volume ingested per lap, although the difference between lesioned and control rats only approached statistical significance ($P < 0.10$). Both the reduction in mean drink size and reduction in lap volume were evident on the day following lesioning and the values were similar to those presented in Table 5.

There were no changes in the circadian rhythm of ingestion; 70-80% of the meals and daily water intake continued to occur at night in all groups. Furthermore, there were only small changes in the temporal relationship of feeding and drinking. In rats with ZI (ventral) lesions there was a significant increase in the amount of drinking which occurred during meals and a decrease in drinking after meals (Figure 14). Similarly, there was an increased probability of meals being interrupted by drinking (Figure 15). The same trends were observed in rats with ZI lesions not extending into the LH, but they did not reach statistical significance ($P < 0.10$). As indicated by Kissileff (1970), however, the amount of drinking considered to occur during meals is somewhat arbitrary, and depends on the criterion selected for separating meals from each other. In this case a simple increase in meal frequency as observed for rats with ZI (ventral) lesions would increase the probability of meals occurring closer together and the chance of two meals being classed as one; the drinking which occurred before or after these two meals

would be considered as occurring during the larger meal. When the amount of drinking occurring during meals was recalculated using a criterion of 5 minutes to separate meals rather than 10 minutes, the difference between groups was reduced (percent of total water intake during meals: control lesions [N = 6], 10.9 ± 5.2%; ZI (dorsal) lesions [N = 7], 10.0 ± 2.5%; ZI (ventral) lesions [N = 8], 17.1 ± 3.8%; $P > 0.20$). It should also be noted that the drinking which occurred during meals was not like the prandial drinking described by Kissileff (1969a) for rats recovered from large LH lesions; less than 30% of the meals were interrupted by drinking (10 min criterion) and very few were interrupted by more than one drink. Mean draft size of the drinks interrupting feeding was 0.74 ± 0.80 ml for rats with ZI (ventral) lesions.

DISCUSSION

The results obtained from the continuous records of feeding and drinking before lesioning are in close agreement with the findings of others (Fitzsimons and LeMagnen, 1969; Kissileff, 1969a), demonstrating a circadian rhythm in ingestion and close temporal relationship between feeding and drinking. Lesions of the ZI produced only minor changes in this pattern.

The immediate effect of making lesions through implanted electrodes was a burst of feeding and drinking behavior.

This may have been caused by irritative stimulation of adjacent structures in the LH involved in the control of food and water intake (Rolls, 1970). Lesions dorsal to the ZI may have induced drinking by similar stimulation of neural elements of the ZI involved in the control of water intake. Transient irritative stimulation of neural structures being damaged may have also contributed to the increased drinking since withholding the water for one hour reduced the water intake. The increased feeding and drinking did not appear to be related to a non-specific increase in general activity, since the rats were relatively quiescent during the period when food and water were withheld.

The transient depression of water intake in rats after lesions of the ZI and the occurrence of meals not accompanied by drinking are consistent with the hypothesis that secondary factors are important in determining the normal pattern of water intake (Fitzsimons and LeMagnen, 1969) and that lesions of the ZI disrupt secondary drinking. The resumption of drinking several hours later could be interpreted as a response to signals of dehydration. However, it would be necessary to test the drinking response to primary deficit signals during the initial period of adipsia to confirm that there was not a transient depression of all water intake. It is unlikely that the adipsia was caused by a general motor disability since eating occurred during this time and "attempts" to drink were not observed.

The finding that normal patterns of feeding resume shortly after lesions restricted to the ZI is consistent with the observation that electrical stimulation of this region elicits drinking behavior but not eating (Huang and Mogenson, 1972). This provides further evidence that neural elements in this region are specifically involved in the control of drinking behavior. Lesions which extended into the LH did cause a temporary aphagia, as observed by Huang and Mogenson (1974), but since damage to the LH was minimal, daily food intake recovered within a few days. The rats remained hypodipsic, however, due to the extensive damage to the ZI.

Following the temporary depression of drinking behavior, rats with lesions of the ZI resumed patterns of food and water intake that were not significantly different from those before lesioning or those of controls. A slight increase in the amount of water ingested during meals was observed, but this may have been due to subtle changes in meal pattern, and was more evident in rats in which lesions extended into the LH.

Walsh and Grossman (1973), observing that lesions of the ZI attenuated drinking during food deprivation, suggested that rats with lesions of the ZI may drink only to facilitate the ingestion of dry food and engage in the prandial style of drinking reported for rats recovered from LH lesions and rats with salivary deficits. Prandial drinking is character-

ized by the frequent interruption of feeding by the ingestion of small drafts of water averaging 0.2 ml in size and seldom greater than 0.5 ml (Kissileff, 1969a). In the present experiment, few meals were observed to be interrupted by drinking, even fewer by more than one drink, and the mean draft size during feeding was greater than 0.5 ml. It is concluded that rats with lesions of the ZI do not engage in prandial drinking.

Several authors have emphasized the importance of secondary factors in determining daily patterns of feeding and drinking (Fitzsimons and LeMagnen, 1969; Collier et al., 1972; Fitzsimons, 1973; Oatley, 1973). The failure of lesions of the ZI to permanently disrupt normal patterns of ingestion, however, does not necessarily mean that secondary controls of water intake remain intact. Two other recent reports have noted that manipulations which reduce daily water intake to minimal requirements for fluid balance do not attenuate the drinking occurring with feeding (guinea pigs bar pressing for water, Hirsch and Collier, 1974; rats bar pressing for intravenous injections of water, Rowland and Nicolaïdis, 1975). Primary signals of dehydration arise during feeding due to the absorption of solute and movement of fluids into the gastrointestinal tract (Lepkovsky et al., 1957; Novin, 1962). Under stable environmental conditions and when water is available ad libitum, drinking often occurs in anticipation of these signals of water deficit

(Oatley and Toates, 1969) and animals can learn to associate the act of feeding with subsequent needs for water (Fitzsimons and LeMagnen, 1969). Nevertheless, even in the absence of secondary controls, drinking would tend to occur in close temporal relationship to feeding. This has been an important factor in the controversy over whether meal-associated drinking is under primary or secondary control (Kissileff, 1973; Oatley, 1973) and indicates that the simple observation of patterns of intake is not adequate for differentiating between the two; more detailed measurements of the time course of fluid shifts with eating, like those described by Oatley and Toates (1969), are necessary to settle this issue. Even the drinking that occurs prior to a meal may not be a good criterion to use for demonstrating secondary control since there is evidence to suggest that the act of drinking and subsequent changes in serum osmolality may disinhibit feeding (Kakolewski and Deaux, 1970).

The most characteristic feature of the hypodipsia after lesions of the ZI was a reduction in mean drink size. This reduction was evident shortly after lesioning and was significantly correlated with the reduction in daily water intake. There is evidence that in the intact rat positive feedback mechanisms may participate to maintain drinking once it has begun (Oatley, 1973) and Kissileff (1973) has proposed that normally drinking is terminated only after the ingestion of a fixed minimal volume of water (0.5-1.0 ml), regardless of the

initiating stimulus. Thus, the observation that rats with lesions of the ZI restrict their total daily water intake to actual fluid requirements, may also be true of individual drinks; drinking may occur more precisely in relation to the intensity of the signals of dehydration.

Although rats with lesions of the ZI drank less water than controls, they did not lap at the drinking spout less frequently. The implication of this finding is that rats with lesions of the ZI are less efficient at drinking and hence must work more than controls to obtain a given volume of water. Several studies have reported that when the effort required to obtain water is increased, water intake is reduced to minimal fluid requirements (Morrison, 1968; Hirsch and Collier, 1974). This raises the possibility that the reduction in daily water intake and attenuation of secondary drinking in rats with lesions of the ZI is not due to a disruption of neural pathways specifically involved in the control of secondary drinking but is subsequent to a less specific constraint on fluid ingestion. This is investigated in the experiment presented in Section 3.5.

3.5 General Inhibition of Drinking after Lesions of the Zona Incerta

To summarize the results of the previous experiments, daily water intake in rats with lesions of the ZI is reduced towards minimal requirements for maintaining homeostasis. In Section 3.3 it was proposed that this was due to the attenuation of secondary drinking. The results of Section 3.4, however, indicate that lesioned rats may have greater difficulty obtaining water from the drinking spout. Several manipulations which make drinking more difficult (bar pressing for water, Morrison, 1968) or aversive (unpalatable solutions, Pfaffman, 1960) also reduce drinking in excess of needs. Therefore, it is important to determine whether the reduction in daily water intake after lesioning is due to a disruption of neural elements specifically involved in the control of secondary drinking or results from a more general inhibition of water intake such as a subtle impairment in the ability to drink.

If lesions of the ZI cause a general inhibition of water intake, then the drinking response to signals of water deficit should also be affected. The results presented in Section 3.1 demonstrate that lesioned rats drink as much water as controls to intracellular and extracellular dehydration, but these testing procedures may not reveal subtle

impairments. Reduction of water intake is not a linear function of increasing work load, and animals will tolerate some increase in effort to meet water requirements for fluid balance, although they will not engage in excess drinking (Morrison, 1968; Hirsch and Collier, 1974). If lesions of the ZI do place some non-specific constraint on water intake, however, the drinking response of lesioned rats to signals of water deficit should be more sensitive to additional inhibitory influences than that of controls. In the present experiment water intake after water deprivation was determined for lesioned and control animals when the drinking solution was made increasingly bitter by adding quinine. The effect of sweetening the drinking solution by adding saccharin was also investigated, to compare the effect of a facilitatory influence on the intakes of lesioned and control rats.

In the second part of this experiment the drinking response of lesioned rats to cellular dehydration was re-examined. Burke et al. (1972) have demonstrated that the drinking response to injections of hypertonic saline is particularly sensitive to inhibitory influences. In Section 3.1 it was reported that lesions of the ZI do not reduce the drinking response to saline injections, but recently Walsh and Grossman (1974) have observed such a deficit after more anterior lesions. Although a difference in lesion site may explain this discrepancy (Section 3.1), it is noteworthy that Walsh and Grossman injected solute loads

twice as large as those used in Section 3.1 to induce cellular dehydration (personal communication). In the present experiment water intakes after the injection of low and high solute loads were compared in lesioned and control rats. The results are discussed in relation to a non-specific inhibition of drinking behavior after lesions of the ZI.

METHODS

Thirty rats were maintained for 9 days with food and water provided ad libitum. Then 21 of the rats received bilateral lesions in the region of the ZI and 9 were sham lesioned. Eight days after surgery the rats were placed on a daily schedule of 23.5 hr water deprivation; each day the food was removed, the rat was weighed, returned to its cage and given 1/2 hr access to distilled water. Food was returned at the end of the 1/2 hr session. After 10 days' adaptation to the schedule, presentation of the various solutions to be tested was begun. The solutions were provided in place of the distilled water in the daily 1/2 hr sessions; days on which solutions were presented were separated by at least 2 days in which distilled water was presented. The effects of adding quinine monohydrochloride (Sigma Chem. Co., Mo.) to the drinking water was investigated first. The order of presentation of concentrations of quinine was randomly determined and was as follows, in g/100 ml:

:1, .001, .0001, .05, .005,

.01, .002, .0005, .0002, and .02.

At the conclusion of the quinine tests the following sodium saccharin (Sigma Chem. Co., Mo.) solutions were presented (g/100 ml):

.0001, .01, .1, .001, 1.0, .05, .5, and .2.

At the end of the test schedule the rats were returned to ad libitum food and water for 10 days to re-examine ad libitum intakes.

During the next two weeks the rats received two injections of hypertonic saline and two control injections of isotonic saline to investigate the effect of degree of cellular dehydration on the water consumed by lesioned and control animals. In the first week the water intake was determined for 1/2, 1 and 2 hr following ip injections of 2 M NaCl (2 ml/kg body weight) and following injections of equivalent volumes of isotonic saline. One week later the drinking response to sc injections of 0.5 M NaCl (4 ml/kg body weight) and to isotonic saline was tested. At the end of these tests the rats were sacrificed and the site and extent of the lesions were determined histologically.

RESULTS

As observed in the previous experiments, when water was available ad libitum, rats with lesions of the ZI drank significantly less water than controls both immediately after the lesions and at the end of the sequence of tests ($P < 0.01$, Table 6). There were no significant differences between groups in daily food intake or body weight ($P > 0.20$ for all comparisons).

Intake of Quinine and Saccharin Solutions

All rats readily adapted to the 23.5 hr water deprivation schedule; there were no differences between groups in food intake or body weight throughout the sequence of tests. As reported in Section 3.1, rats with lesions of the ZI drank less water after water deprivation than sham-lesioned controls ($P < 0.01$, Figure 17).

The effect of adding quinine to the drinking water is presented in Figure 17. Analysis of variance demonstrated a significant decline in fluid intake with increasing quinine concentration ($P < 0.01$) and a significant difference between the intakes of lesioned and control rats ($P < 0.01$). There was also a significant interaction between group and quinine concentration ($P < 0.01$), which is evident in the difference in the shape of the two curves in Figure 17.

TABLE 6. DAILY WATER INTAKE OF RATS BEFORE AND AFTER LESIONING

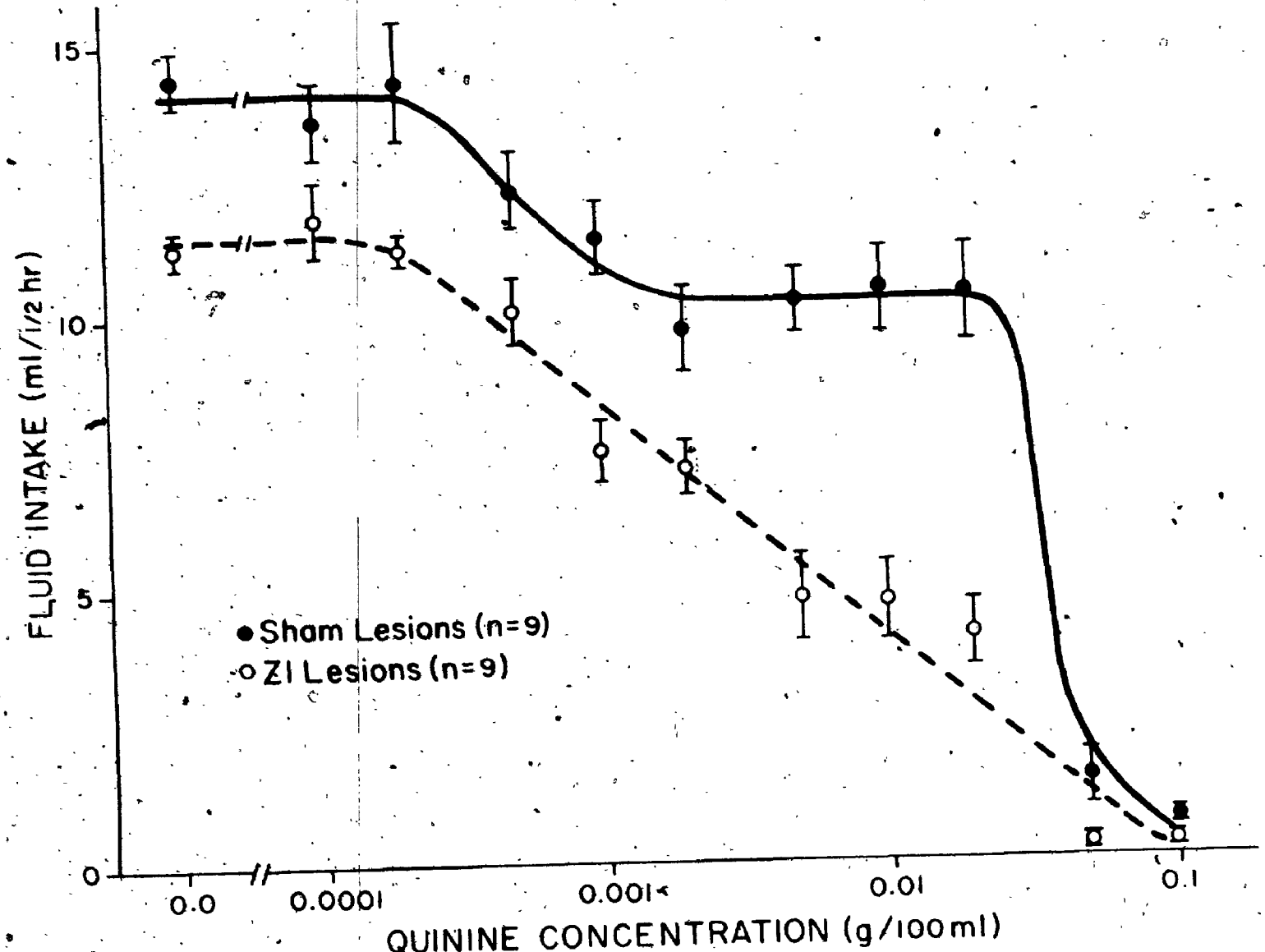
Group	Before Lesioning (ml/day)	1 Week after Lesioning (ml/day)	11 Weeks after Lesioning (ml/day)
Sham Lesions (N = 9)	19.6 ± 0.8	21.9 ± 1.0	22.9 ± 0.8
Dorsal Lesions (N = 3)	20.0 ± 1.1	20.0 ± 0.6	22.2 ± 1.0
ZI Lesions (N = 9)	20.8 ± 1.7	17.6 ± 0.6*	18.6 ± 0.5*

Values are means ± SEM calculated from the average of 5 days for each rat.

* significantly different from the value for the sham lesion group, $P < 0.01$.

FIGURE 17

Effect of concentration of quinine in the drinking water on the volume ingested (mean ± SEM) by rats after 23.5 hours water deprivation. Quinine concentration is plotted on a logarithmic scale. A regression line was fitted to the data points for rats with lesions of the zona incerta (ZI). The curve for the sham-lesioned group was fitted by eye.


15
10
5
0
FLUID INTAKE (ml/1/2 hr)
● Sham Lesions (n=9)
○ ZI Lesions (n=9)
0.0
0.0001
0.001
0.01
0.1
QUININE CONCENTRATION (g/100ml)

The lowest concentration of quinine for which a reduction in water intake was observed was identical for the two groups. At concentrations greater than this, rats with lesions of the ZI exhibited a steady decline in intake closely fitting a linear regression line defined by

$$Y = -4.1 (\log X - 1.0)$$

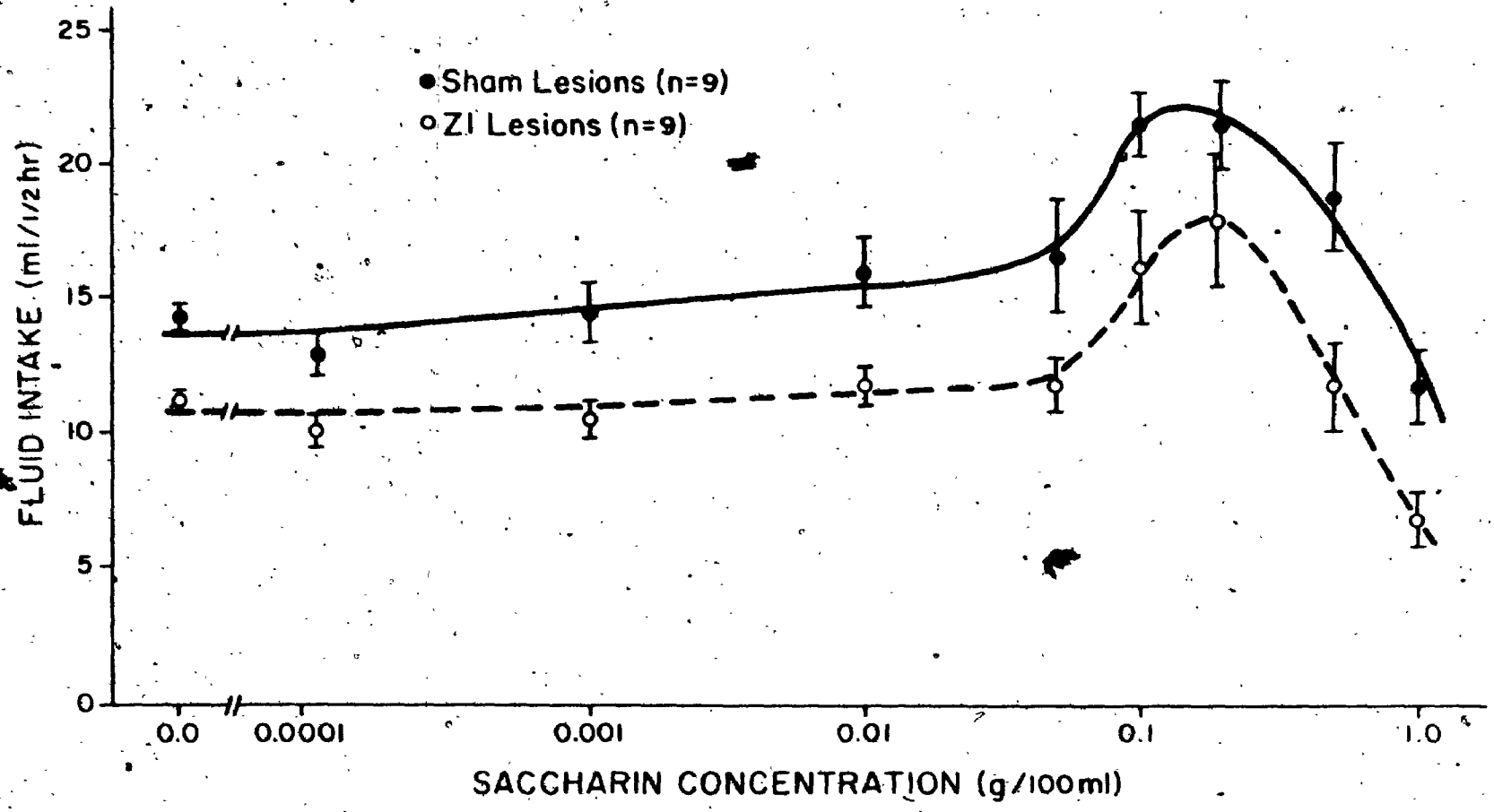
where Y = fluid intake and X = quinine concentration in gm/100 ml ($r = 0.98$, $P < 0.001$). Control rats, however, exhibited a second plateau in the reduction of water intake between quinine concentrations of 0.001 and 0.02 gm/100 ml; fluid intakes at these concentrations were not significantly different than those of rats with lesions of the ZI drinking distilled water ($P > 0.20$, Figure 17).

The effect of adding saccharin to the drinking solution is presented in Figure 18. Initially there was a significant increase in fluid intake with increasing saccharin concentration for both groups ($P < 0.01$) but rats with lesions of the ZI continued to drink significantly less than sham lesioned rats ($P < 0.05$). At higher concentrations saccharin solutions became increasingly aversive, as observed by others (e.g., Corbit and Luschei, 1969).

To assess the relationship between the effects of lesions of the ZI on ad libitum water intake and on the response to the solutions, correlation coefficients were calculated to compare the intakes of distilled water, 0.01% (gm/100 ml) quinine solution and 0.1% saccharin solution

FIGURE 18

Effect of concentration of saccharin in the drinking water on the volume ingested (mean ± SEM) after 23.5 hours water deprivation. Saccharin concentration is plotted on a logarithmic scale. Curves were fitted by eye.


Sham Lesions (n=9)
ZI Lesions (n=9)
FLUID INTAKE (ml/1/2 hr)
25
20
15
10
5
0
0.0
0.0001
0.001
0.01
0.1
1.0
SACCHARIN CONCENTRATION (g/100ml)

after water deprivation, and ad libitum water intake at the conclusion of the sequence of tests. All animals were included in the calculation, including rats receiving asymmetric lesions. There were significant correlations between all of the variables (Table 7). A scatter diagram illustrating the relationship between 0.01% quinine intake and ad libitum water intake is presented in Figure 19.

Water Intake in Response to Cellular Dehydration

The results of the cellular dehydration tests are presented in Figure 20. Water intake for each rat was calculated as the difference in intake following the injection of hypertonic saline and that following the control injection of isotonic saline. Both lesioned and control rats drank a significant amount of water within 1/2 hr after the sc injection of the lower solute load ($P < 0.01$, Figure 20B). As observed in Section 3.1, when a similar solute load was injected ip, the water intakes of the two groups were not significantly different from each other (Figure 20B). After the injection of the higher solute load, however, rats with lesions of the ZI drank significantly less than controls ($P < 0.01$, Figure 20A). Furthermore, the drinking response of lesioned rats was delayed; they did not drink more water in the first 1/2 hr after the hypertonic saline injection than they did after the control injection ($P > 0.20$).

TABLE 7. CORRELATION COEFFICIENTS: INTAKE OF SOLUTIONS AFTER WATER DEPRIVATION AND AD LIBITUM WATER INTAKE

Variables	r	P
0.01% Quinine vs. Water (dep.)	0.61	< 0.01
0.1% Saccharin vs. Water (dep.)	0.55	< 0.01
0.01% Quinine vs. 0.1% Saccharin	0.47	< 0.01
Water (dep.) vs. Water (ad lib.)	0.43	< 0.05
0.01% Quinine vs. Water (ad lib.)	0.55	< 0.01
0.1% Saccharin vs. Water (ad lib.)	0.52	< 0.01

0.01% Quinine, 0.1% Saccharin and Water (dep.) refer to intakes of these solutions after 23.5 hr water deprivation.

Water (ad lib.) refers to ad libitum water intake at the conclusion of the testing sequence (see text, Section 3.5).

N = 30 for all comparisons.

FIGURE 19

Scatter diagram illustrating the relationship between the volume of 0.01% (g/100 ml) quinine solution ingested after 23.5 hours water deprivation and ad libitum water intake in rats with lesions of the zona incerta (ZI) and sham-lesioned controls. A linear regression line is fitted to the data points (see Table 7).

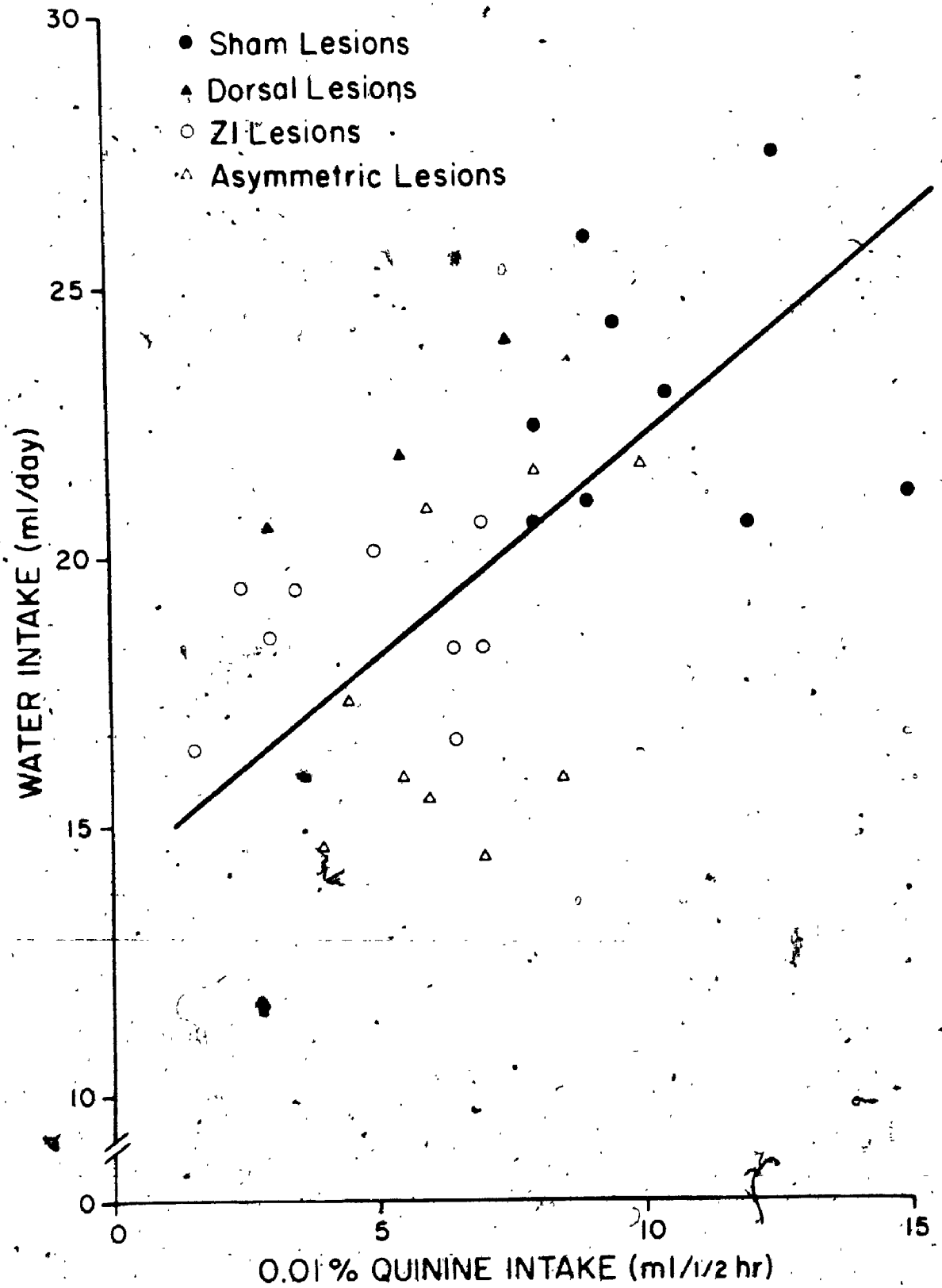
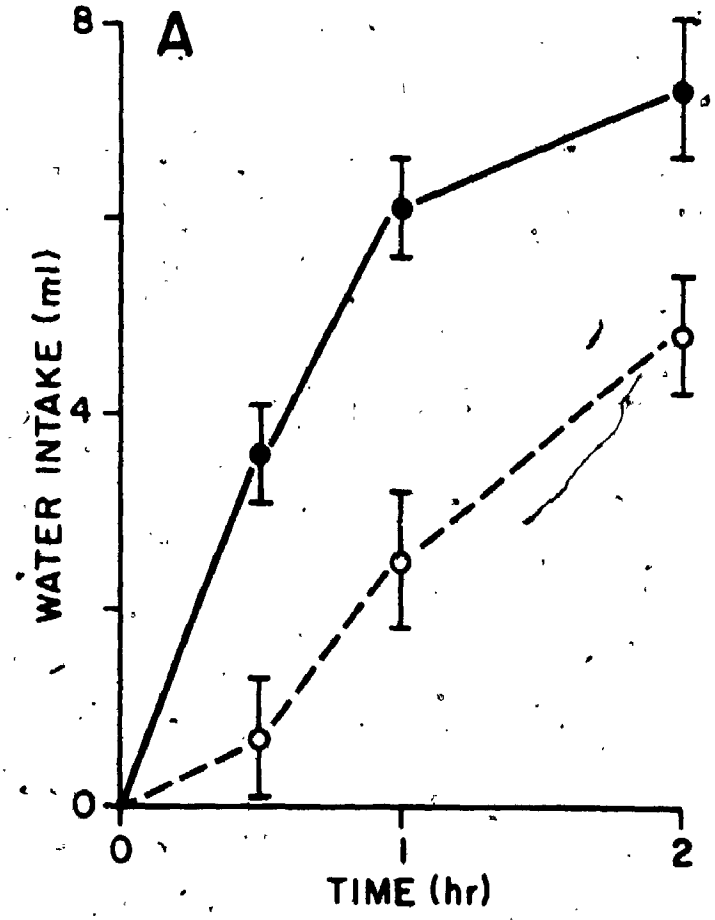
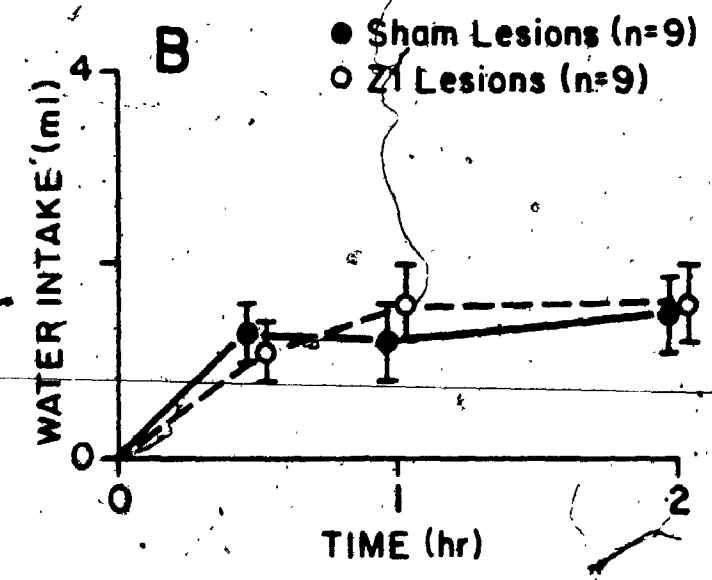

Sham Lesions
Dorsal Lesions
ZI Lesions
Asymmetric Lesions
WATER INTAKE (ml/day)
30
25
20
15
10
0
0
5
10
15
0.01% QUININE INTAKE (ml/1/2 hr)

FIGURE 20

Cumulative water intake in rats with lesions of the zona incerta (ZI) and sham-lesioned controls, induced by the injection of low (B, 0.5 M NaCl, 4 ml/kg) and higher (A, 2 M NaCl, 2 ml/kg) solute loads. Water intake (mean ± SEM) in response to the solute loads was calculated as the difference in the volume of water ingested following injections of the hypertonic saline solutions and that following control injections of isotonic saline. Injections were made at time 0.


A
8
4
0
WATER INTAKE (ml)
0
1
2
TIME (hr)


B
● Sham Lesions (n=9)
○ ZI Lesions (n=9)
4
0
WATER INTAKE (ml)
0
1
2
TIME (hr)

DISCUSSION

In Section 3.1 it was reported that lesions of the ZI do not reduce water intake in response to body fluid deficits. The results of the present experiments, however, demonstrate that the drinking response of lesioned rats to signals of dehydration is more sensitive to inhibitory influences on water intake than that of control animals.

In control rats the effect of adding quinine to the drinking water was not linear. Small increases in quinine concentration reduced fluid intake after water deprivation by 25%, but this lower level of intake was then maintained in spite of increasing bitterness of the drinking solution. A similar "plateau" effect has been observed when increasing work loads (bar presses) are coupled to daily water intake (Morrison, 1968; Hirsch and Collier, 1974), and has been ascribed to the loss of an excessive facilitatory component of drinking behavior and subsequent restriction of intake to minimal fluid requirements for homeostasis. Since the results presented in Sections 3.1 to 3.3 demonstrate that lesions of the ZI also reduce daily water intake to minimal fluid requirements, it is noteworthy that the "plateau" level of intake in control rats in the present experiment is not significantly different from that of lesioned rats drinking distilled water.

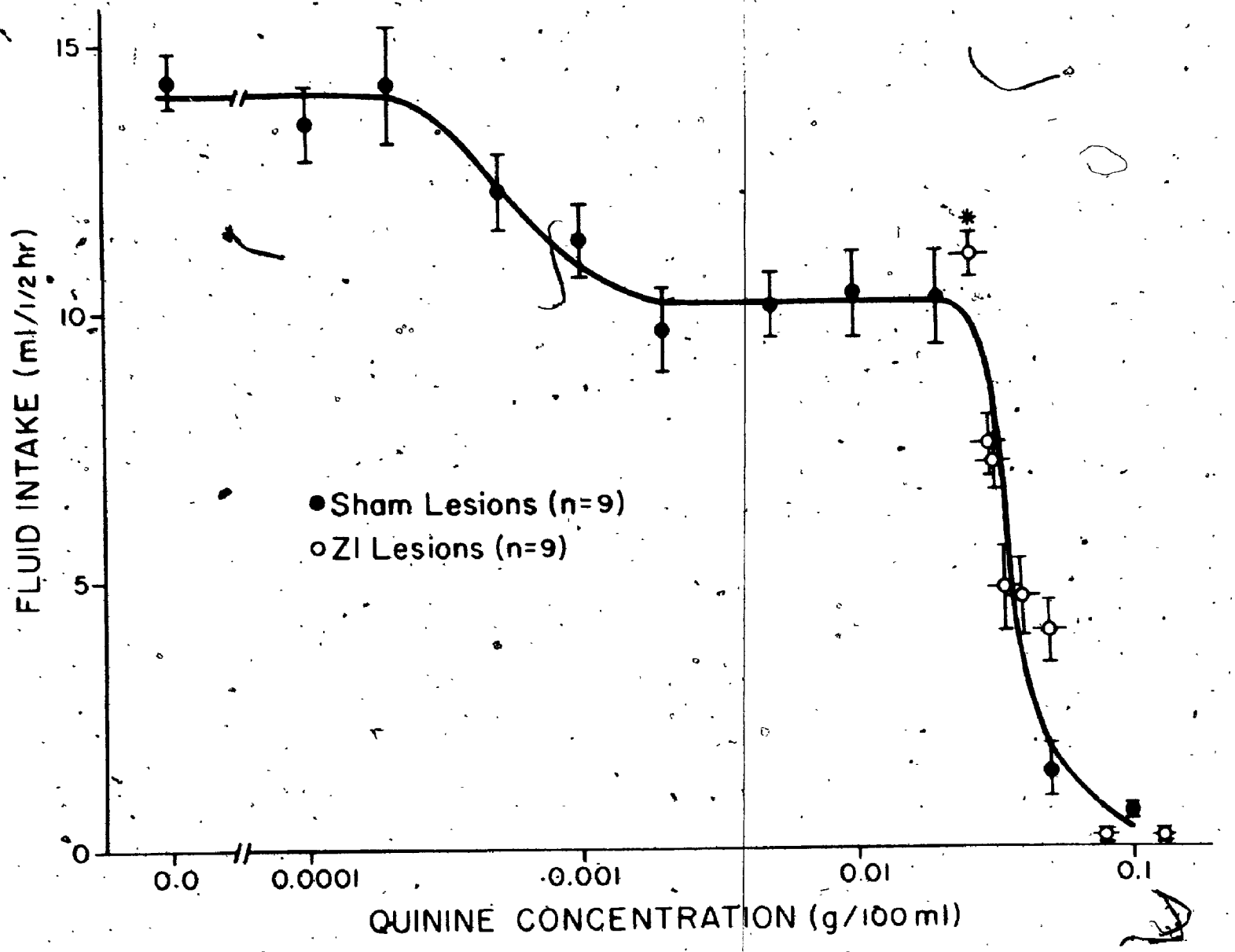
Whereas control rats maintained water intake despite increasing quinine concentration, the intake of rats with

lesions of the ZI was attenuated. Only at higher concentrations was a similar reduction in intake of the control group observed. Although it appears from Figure 17 that the slope of the final decline in intake with increasing bitterness of the solution is different in the two groups, this is an artifact of the logarithmic presentation, since both curves fall over a similar increase in quinine concentration (0.05 gm/100 ml). This can be shown graphically by shifting the curve for lesioned rats to the right along the abscissa by a constant amount (quinine concentration), determined empirically or mathematically from the curves. Such a transformation is presented in Figure 21 for a shift of 0.03 gm/100 ml quinine concentration. The data points for the rats with lesions of the ZI then closely fit the descending portion of the curve for control rats. Thus, the curve for rats with lesions of the ZI in Figure 17 appears as a curve for control rats shifted towards lower quinine concentrations. In other words, a rat with lesions of the ZI may be defined as an intact rat with the addition of some inhibition of drinking equivalent to replacing its drinking water with 0.03 gm/100 ml quinine solution.

Rats with lesions of the ZI also drank less than controls when saccharin was added to the drinking water in both palatable and aversive concentrations. This is consistent with the results for quinine presentation and confirms the existence of an inhibition on water intake after deprivation

FIGURE 21

Transformation of the results presented in Figure 17, illustrating the effect of shifting the data points of rats with lesions of the zona incerta (open circles) 0.03 g/100 ml along the abscissa (note the logarithmic scale). Horizontal error bars represent ± 0.005 g/100 ml. The point marked by the asterisk was calculated from the average intake of distilled water and three lowest quinine concentrations (0.0001-0.005) presented in Figure 17 and is plotted at 0.03 g/100 ml. The curve for the sham-lesioned rats is identical to that presented in Figure 17.


15
10
5
0
FLUID INTAKE (ml/1/2 hr)
●Sham Lesions (n=9)
○ZI Lesions (n=9)
0.0
0.0001
0.001
0.01
0.1
QUININE CONCENTRATION (g/100 ml)

in lesioned rats. Lesioned rats did not exhibit the "finickiness" or increased responsiveness to palatability described for rats with lesions of the septum (Flaherty and Hamilton, 1971) or ventromedial hypothalamus (Stevenson, 1969) which also drink less of bitter solutions, since they did not drink more than controls when the water was sweetened. Furthermore, these results cannot be attributed to reduced taste sensitivity since this would have reduced the response to quinine rather than have enhanced it as shown in Figure 17.

In the second half of this experiment the drinking response of lesioned rats to cellular dehydration was re-examined. Walsh and Grossman (1974), injecting solute loads similar to the larger of those tested here (4 M NaCl, 1 ml/kg, personal communication), reported a deficit in the drinking response to cellular dehydration after lesions of the anterior ZI. In the present experiment it was found that rats with lesions of the ZI drank as much as controls in response to low but not high solute loads. This effect can be interpreted in relation to the findings of the first half of this experiment. The injection of hypertonic solutions is stressful to animals and the sudden rise in body fluid tonicity may have an inhibitory effect on behavior in addition to the excitation of osmoreceptors. Burke et al. (1972) have made the important observation that drinking induced in this way is particularly sensitive to additional

constraints on drinking; making the drinking solution only mildly aversive attenuated the drinking to cellular dehydration but not extracellular dehydration or water deprivation. The results of the first half of the present experiment demonstrate that lesions of the ZI have an effect analogous to making drinking more aversive. Since the stress of hypertonicity after saline injections would increase with higher solute loads, any additional suppression of drinking due to lesioning would become more evident. Therefore, the effect of lesions of the ZI on drinking to hypertonic saline solutions should not be interpreted as a specific effect on neural elements mediating the drinking response to cellular dehydration as suggested by Walsh and Grossman (1974).

In summary, contrary to the earlier findings (Section 3.1), further investigation has demonstrated that lesions of the ZI also have a subtle but significant effect on the drinking response to dehydration. It is concluded from these results that lesions of the ZI cause a general inhibition of water intake in a manner analogous to requiring the rat to drink unpalatable solutions or work to obtain water. Under such conditions animals will drink water to maintain fluid balance but will not engage in excess drinking (Morrison, 1968; Hirsch and Collier, 1974). In the next experiment the nature of the suppression of water intake after lesions of the ZI is investigated.

3.6 Deficit in the Ability to Ingest Fluids after Lesions of the Zona Incerta

It was initially proposed that lesions of the ZI reduced daily water intake and attenuated secondary drinking in rats by disrupting neural elements specifically involved in the control of drinking to secondary cues (Section 3.3). The results presented in Section 3.5, however, indicate that lesions of the ZI produce a more general inhibition of drinking behavior. The purpose of the present experiment was to investigate the mechanism of this inhibition.

It was observed in Section 3.4 that when water was available ad libitum, rats with lesions of the ZI made as many laps at the drinking spout as controls but obtained less water per lap, suggesting that lesioned rats were less efficient at drinking. Several studies have demonstrated that when the effort required to obtain water is increased, daily water intake is reduced to minimal requirements for maintaining water balance (Morrison, 1968; Hirsch and Collier, 1974). Since the reduction in lap volume observed in Section 3.4 only approached statistical significance, the present experiment was conducted to confirm this deficit. It was assumed that if an impairment in the ability to ingest fluids existed, then it would also be evident in the ingestion of fluids other than water, including the ingestion of liquid diet after food deprivation.

METHODS

Apparatus and Testing Procedure

Cages equipped with the drinkometer circuits described in Section 3.4 were used to record drinking in rats in the 1/2 hr following periods of food or water deprivation. One of three solutions was available in the drinking tube: distilled water, 0.005% (gm/100 ml) quinine solution, or liquid diet (1 part Borden's Sweetened Condensed Milk, 2 parts water). Solid food was not available during the 1/2 hr tests. The pattern and number of laps and the volume of fluid ingested, corrected for spillage, were recorded. Following this 1/2 hr period the rats were returned to their home cages where regular diet and water were available ad libitum. Food was available during the water deprivation and yice versa.

General Procedure

Twenty-six rats were maintained as in the previous experiments with food and water available ad libitum. One week after the beginning of the experiment they were adapted to the recording apparatus by placing them in the cages for 1/2 hr for each of 2 days. A pre-operative deprivation and testing sequence was then carried out to adapt the rats to the procedure and the solutions. Two days separated each test. The rats were water deprived for 24 hr and given water to drink, food deprived for 24 hr and given liquid

diet to drink, and water deprived for 24 hr and given liquid diet to drink, in that order.

Five days later, 15 rats were lesioned in the region of the ZI and 7 were sham lesioned. Beginning 8 days after surgery the three tests described for the pre-operative period were repeated twice, first in the same order as above and then in the reverse order. As before, at least 2 days separated each test.

Two more tests were carried out to determine the effect of a shorter water deprivation and the effect of adding quinine to the drinking water on the recorded water intake. The rats were water deprived for 12 hr and given water to drink, and water deprived for 24 hr and given 0.005% quinine solution. These tests were then repeated with the order reversed. The rats were maintained for one additional week with food and water available ad libitum before they were sacrificed and the site and extent of the lesions determined histologically.

RESULTS

As in the previous experiments lesions of the ZI, but not lesions dorsal to the ZI, produced a significant reduction in ad libitum water intake ($P < 0.01$, Table 8). The lesions did not reduce daily food intake or body weight (Table 8).

TABLE 8. AD LIBITUM FOOD AND WATER INTAKES AND BODY WEIGHT FIVE WEEKS AFTER LESIONING

Group	Food Intake (g/day)	Water Intake (ml/day)	Body Weight (g)
Sham Lesions (N = 7)	21.5 ± 0.7	24.1 ± 1.6	397 ± 12
Dorsal Lesions (N = 6)	23.4 ± 1.0	23.3 ± 1.7	428 ± 14
ZI Lesions (N = 9)	23.0 ± 1.2	16.0 ± 0.8*	406 ± 14

Values are means ± SEM calculated from the average of 5 consecutive days for each rat.

* significantly different from the value for the sham lesion group, $P < 0.01$.

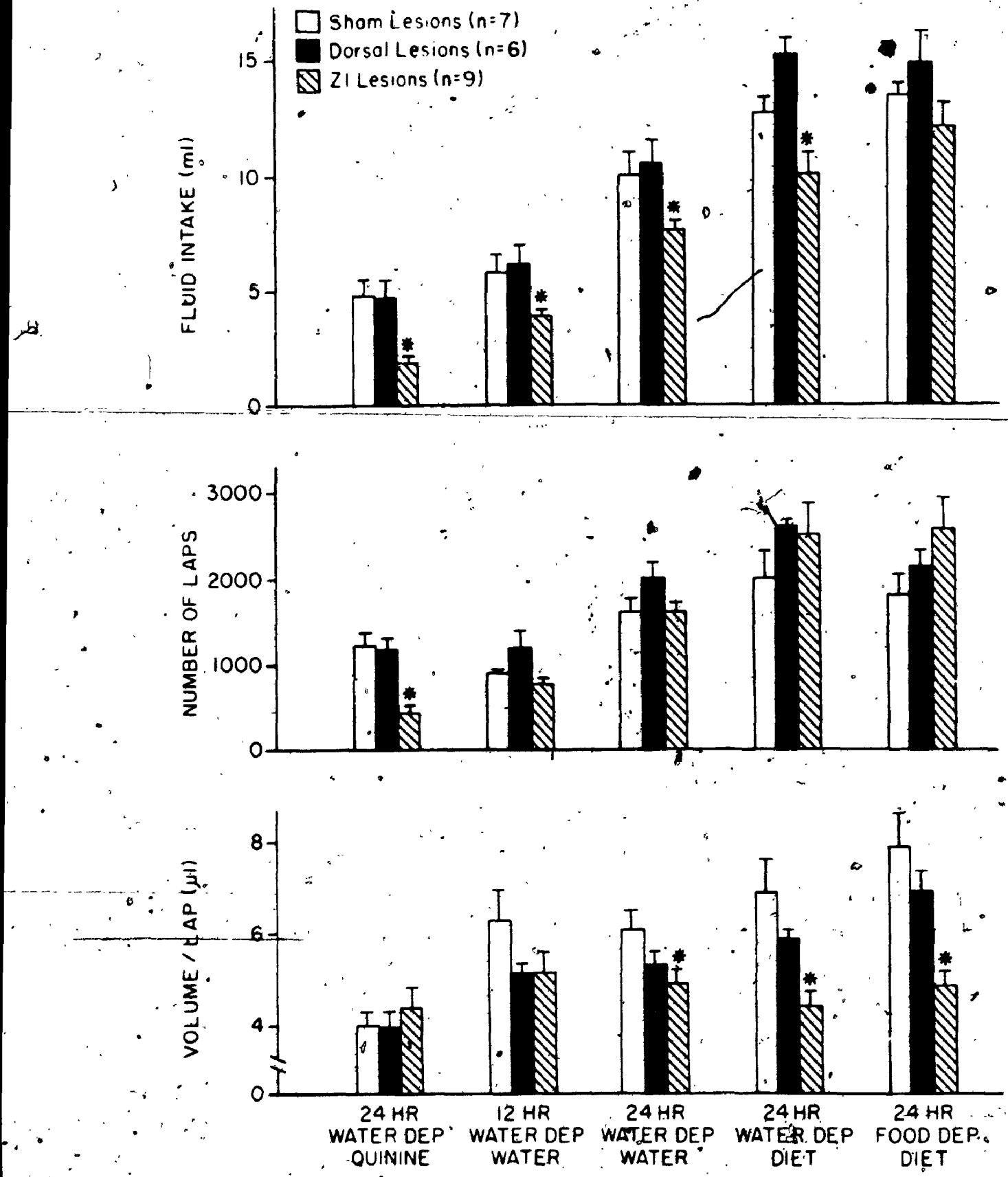
In the pre-operative drinking tests the rats readily drank the water and liquid diet from the recording apparatus. There were no significant differences between groups in intake, number of laps, or lap volume. The results of the drinking tests after lesioning are presented in Figure 22. All rats approached the spout and began licking within one minute of being placed in the recording cage. Except when drinking quinine solution, 80-95% of the drinking was completed within the first 15 minutes with only short pauses between drinks. When the rats were drinking the quinine solution bouts of licking were shorter and were more dispersed throughout the 1/2 hr session.

As observed in the previous experiments rats with lesions of the ZI drank significantly less than controls after water deprivation, regardless of whether they were drinking water, quinine solution or the liquid diet (Figure 22). They did not drink significantly less liquid diet than controls after food deprivation ($P > 0.20$). Except when drinking quinine solution, however, rats with lesions of the ZI did not lick the drinking spout less than controls but obtained less fluid per lap. This was most evident when rats were ingesting the liquid diet but also occurred to a lesser extent when water was available (Figure 22). Lesioned rats did not obtain less volume per lap as compared to controls when drinking quinine solution, but made fewer laps at the spout. Rats with lesions dorsal to the ZI

FIGURE 22

Total fluid intake, number of laps at the drinking spout, and the volume of fluid obtained per lap, after water or food deprivation in rats with lesions of the zona incerta (ZI) or sham lesions. The length of deprivation (dep) and solutions tested are described at the bottom of the figure. Bars represent mean ± SEM.

* significantly different from the value for the sham-lesioned group, $P < 0.05$


Sham Lesions (n=7)
Dorsal Lesions (n=6)
ZI Lesions (n=9)
FLUID INTAKE (ml)
0
5
10
15
NUMBER OF LAPS
0
1000
2000
3000
VOLUME / LAP (µl)
0
4
6
8
24 HR WATER DEP QUININE
12 HR WATER DEP WATER
24 HR WATER DEP WATER
24 HR WATER DEP DIET
24 HR FOOD DEP. DIET

tended to obtain less volume per lap than sham-lesioned controls, but this difference did not reach statistical significance. Furthermore, lap volumes in these rats were slightly lower than those of controls before lesioning.

The effect of the different treatments (water or food deprivation, water, quinine, or liquid diet) on the three parameters of intake presented in Figure 22 was also examined (Table 9). There was a significant difference in the volume of fluid ingested across treatments in all 3 groups, as expected from the difference in deprivation periods and palatability of the solutions. This difference in total intake was also reflected in the number of laps. Thus, as reported by Stellar and Hill (1952), the animals increased fluid intake by engaging in longer periods of licking. However, as shown in the bottom panel of Figure 22 and Table 9, sham lesioned rats and rats with lesions dorsal to the ZI also varied lap volume across treatments, indicating that an increase in the amount obtained with each lick also contributed to an increase in total intake. Lap volume did not vary across treatments in rats with lesions of the ZI ($P > 0.50$), but remained at the lowest level observed in control animals.

Lap rate was determined from the records by dividing the total number of licks by the time spent drinking. As reported by others (Stellar and Hill, 1952; Corbit and Luschei, 1969), rate of licking did not vary significantly

TABLE 9. SUMMARY OF ANALYSES OF VARIANCE: EFFECT OF NATURE AND LENGTH OF DEPRIVATION AND DRINKING SOLUTION ON FLUID INTAKE, NUMBER OF LAPS AND LAP VOLUME*

	Degrees of Freedom	F	P
Fluid Intake			
Sham lesions	4, 30	34.26	< 0.01
Dorsal lesions	4, 25	24.27	< 0.01
ZI lesions	4, 40	41.57	< 0.01
Number of Laps			
Sham lesions	4, 30	6.80	< 0.01
Dorsal lesions	4, 25	17.54	< 0.01
ZI lesions	4, 40	24.15	< 0.01
Volume/Lap			
Sham lesions	4, 30	5.93	< 0.05
Dorsal lesions	4, 25	13.35	< 0.01
ZI lesions	4, 40	0.80	> 0.5

* Comparison across treatments of data presented in Figure 22.

across treatments. Furthermore, lap rate was not significantly different between groups (average for all tests, sham lesions (N = 7), 5.63 ± 0.20 laps/sec; dorsal lesions (N = 6), 5.75 ± 0.08 laps/sec; ZI lesions (N = 9), 5.56 ± 0.16 laps/sec).

To determine whether the effects of lesions on lap volume and on total intake were related, correlation coefficients were calculated (Table 10). Since the greatest difference in lap volume was observed when rats were drinking liquid diet, these values were used in the calculations. Lap volume showed a significant correlation with ad libitum water intake and drinking after water deprivation. As demonstrated in Section 3.5, there was also a significant correlation between the intake of quinine solution and these parameters (Table 10).

DISCUSSION

The results confirm the finding reported in Section 3.4 that rats with lesions of the ZI obtain less water per lap at the drinking spout than control animals. An unexpected finding of this experiment was that lap volume varied across treatments in control rats.

The volume of fluid obtained per lap was slightly increased when control rats were ingesting liquid diet and decreased when they were drinking quinine solution. Previous studies have reported that lap volume,

TABLE 10. CORRELATION COEFFICIENTS: LAP VOLUME, INTAKE OF SOLUTIONS AFTER DEPRIVATION, AND AD LIBITUM WATER INTAKE

Variables	r	p
Lap Volume (liq. diet, 24 hr food dep.) vs:		
Lap Volume (liq. diet, 24 hr water dep.)	0.91	<0.01
Water Intake (24 hr water dep.)	0.52	<0.05
Water Intake (12 hr water dep.)	0.60	<0.01
Water Intake (ad libitum)	0.63	<0.01
Quinine Intake (24 hr water dep.)	0.47	<0.05
Quinine Intake (24 hr water dep.) vs:		
Liq. Diet Intake (24 hr water dep.)	0.62	<0.01
Water Intake (24 hr water dep.)	0.80	<0.01
Water Intake (12 hr water dep.)	0.81	<0.01
Water Intake (ad libitum)	0.66	<0.01

Variables refer to treatments and measurements presented in Fig. 22 (see text, Section 3.6). N = 22 for all comparisons.

like lap rate, is relatively constant and that variations in intake are achieved primarily by varying the duration of bouts of licking (Stellar and Hill, 1952; Corbit and Luschei, 1969). Allison (1971), however, has demonstrated that modifications of the components of the licking response may also occur, including variations in the duration of each lap, the interval between laps, and lap volume. Lap volume has been reported to vary with mechanical factors such as rat size, size of the spout opening, and distance the tongue must be extended to reach the spout (Corbit and Luschei, 1969). In the present experiment, the slightly greater viscosity of the liquid diet may have contributed to the higher lap volume for this solution, but an increased lap volume was not observed in rats with lesions of the ZI drinking liquid diet, nor can viscosity differences explain the reduction in lap volume with quinine solutions. Instead, the difference in lap volume across treatments may reflect a tongue and oropharyngeal response to palatability. Evidence that palatability may directly influence activity of the tongue has recently been reported by Davis (1973).

Corbit and Luschei (1969) have recorded the drinking of saccharin solutions after 16 hr water deprivations as well as the drinking of water after 24-96 hr water deprivations, but report that lap volume did not correlate

with the stimulus to drink. However, it is not indicated how this correlation was calculated; if it was computed on the basis of total fluid intake, including response after long periods of water deprivation, an effect due to taste may have been obscured. It is noteworthy that they report a range of lap volumes comparable to that observed here. (4-8 µl).

Lap volume did not vary across treatments in rats with lesions of the ZI but was maintained at a level equal to the lowest intake observed for controls. This cannot be explained as a failure to detect differences in palatability or other characteristics of the drinking solutions since total fluid intake and number of laps did vary. A reduction in lap volume was also observed when lesioned rats were licking liquid diet after food deprivation, demonstrating that the deficit is not related to the motivation to drink but to the motor act of ingesting fluids. The results confirm the finding of reduced lap volume in lesioned rats when water was available ad libitum (Section 3.4) and support a hypothesis that lesions of the ZI produce an impairment in the ability to ingest fluids.

It is unlikely that the reduced lap volume per se (and resulting inefficiency in drinking) can account entirely for the reduced fluid intake after lesioning; lesioned rats did not exhibit lower lap volumes than controls when drinking quinine solution, yet made signifi-

cantly fewer laps and ingested less solution. However, the effect of lesions of the ZI on lap volume may be symptomatic of an impairment in the act of licking and swallowing that also increases the effort associated with each lap. Thus the reduced intake of quinine in lesioned rats could be explained as the addition of inhibitory influences (bitter solution plus increased effort) as described in Section 3.5. A sensory deficit and loss of facilitatory feedback related to ingestion may also contribute to the inhibition of drinking; this is discussed further in Section 4.1.

Although rats with lesions of the ZI obtained 55% less fluid per lap than controls, they did not ingest less liquid diet after food deprivation. This is consistent with the earlier observation (Section 3.2) that lesions of the ZI do not reduce food intake when liquid diet is available ad libitum, and can be related to the finding that rats will accept large work loads to maintain normal levels of food intake (Collier et al., 1972). It is uncertain whether lesions of the ZI also impair the ingestion of solid food, although no difference in the time spent eating by lesioned and control rats was observed in Section 3.4.

In conclusion, the results of this experiment demonstrate that lesions of the ZI cause an impairment in the ability of rats to ingest fluids. They suggest

that lesions of the ZI reduce ad libitum water intake and attenuate secondary drinking by increasing the effort required to obtain water.

CHAPTER 4. GENERAL DISCUSSION AND SUMMARY

4.1 General Discussion

Although the initial findings of these experiments indicated that lesions of the ZI caused a specific attenuation of secondary drinking (Section 3.3), subsequent experiments have demonstrated a subtle but significant deficit in the drinking response to dehydration as well. Further investigation has shown that rats with lesions of the ZI obtain less fluid per lap at the drinking spout than controls, suggesting an impairment in the ingestion of fluids. Thus, a simple explanation for the reduction in ad libitum water intake after lesions of the ZI is that lesioned rats have greater difficulty obtaining water than controls and as a consequence engage in less drinking. The results of several studies have shown that when the effort required to obtain water is increased, daily water intake is reduced (Morrison, 1968; Hirsch and Collier, 1974). Laboratory rats normally drink in excess of daily water requirements for fluid homeostasis (Dicker and Nunn, 1957) and reductions in intake can be partially compensated for by increased water retention.

The failure of lesions of the ZI to affect water intake in response to signals of dehydration can be

related to the non-linear relationship between water intake and increasing inhibition on drinking (Section 3.5). Except for an initial attenuation of excessive components of intake, animals will tolerate increases in the effort (Morrison, 1968) or aversiveness (control rats, Section 3.5) associated with drinking in order to maintain body fluid balance. A similar interpretation can be applied to the failure of lesions to reduce daily food intake. Although it is uncertain to what extent the impairment evident in the ability to lap fluids affects the ingestion of solid food, it was observed that lesioned animals did not ingest less liquid diet than controls (Sections 3.2, 3.3, and 3.6). Unlike water intake, however, the intake of food is normally more precisely regulated to actual requirements (Mayer, 1967) and rats cannot reduce food intake without compromising energy balance. Therefore, it is not surprising that rats will tolerate increases in the effort required to obtain food without reducing intake (Collier et al., 1972).

Impairments in Oropharyngeal Function

The specific nature of the deficit in the ability of lesioned rats to ingest fluids remains to be determined. Impairments in oropharyngeal function have been reported after ablation of other brain structures in rats. For example, Castro (1972) has demonstrated

deficits in tongue extension after frontal cortex damage, but notes that the rats remained capable of lapping water from a drinking spout. However, deficits in the ability to drink from Richter tubes after lesions of the globus pallidus (Levine and Schwartzbaum, 1973) and the substantia nigra (Marshall et al., 1974) have been reported. Projections to the ZI from both of these areas have been described (Johnson and Clemente, 1959; Shute and Lewis, 1967; Tsubokawa and Sutin, 1972; Jacobowitz and Palkovits, 1974) and efferents from the ZI to the globus pallidus have also been noted (Shute and Lewis, 1967; Huang and Mogenson, 1972; Jacobowitz and Palkovits, 1974). The reciprocal connections of the ZI with the globus pallidus may be particularly relevant to the results of the present study, since Lidsky and co-workers (1975) have recently found that a large proportion (95%) of pallidal units appears to be involved in the processing of sensory information related to the ingestion of fluids in cats. They propose that it is damage to these mechanisms that is responsible for the disruption of ingestive behavior after pallidal lesions (see Levine and Schwartzbaum, 1973). The studies of Zeigler (1973) and Zeigler and Karten (1974) have demonstrated the importance of tactile sensitivity in the control of oropharyngeal musculature.

In this regard, it is noteworthy that various oral afferents project to the ZI. Second order trigeminal fibers have been reported to terminate in the ZI in the region lesioned in the present study (Smith, 1973) and may mediate somatosensory information related to licking and swallowing. Short-latency evoked responses have been recorded in the ZI following mechanical stimulation of the face and intraoral structures of the cat (Darian-Smith, 1964). In the rat, fibers also project to the ZI from a region of the dorsal pons receiving gustatory input from the nucleus of the solitary tract (Norgren and Leonard, 1973). In addition, the ZI has reciprocal connections with the midbrain tegmentum (Johnson and Clemente, 1959; Lindsley et al., 1967; Chi, 1970; Huang and Mogenson, 1972) which may also be involved in the integration of sensory information. Thus, the ZI is in a position to receive oropharyngeal input of several modalities and may participate in the control of oral motor activity through the integration of sensory feedback.

In the past, drinking in the rat has been described as a pre-programmed reflexive response, controlled primarily in an on-off manner (Stellar and Hill, 1952; Corbit and Luschei, 1969). There is evidence, however, that variations in the individual components of this response, including changes in the duration of each lick, the interval between licks, and lick-volume, may also participate in the control of

ingestion (Allison, 1971; Davis, 1973; control rats, Section 3.6). Since lesions of the ZI do not cause a major disruption of licking activity, it is unlikely that this region is involved in the basic reflex response, but may participate in the modulation of individual components such as lap volume. Lap volume of lesioned rats in the present study was lower than that of controls (Sections 3.4, 3.6) and did not vary with the nature of the drinking solution (Section 3.6). In the intact rat, modulation of the licking reflex may involve integration of oropharyngeal information related to taste and tactile properties of the drinking solution with the state of water balance and secondary influences on water intake. Oral afferents to the ZI as well as hypothalamic and limbic interconnections (Section 1.3) may be important in this regard. The influence of the ZI on ingestive reflexes could be exerted via projections to the globus pallidus (Shute and Lewis, 1967; Huang and Mogenson, 1972; Jacobowitz and Palkovits, 1974) or through direct tegmental connections (Johnson and Clemente, 1959; Huang and Mogenson, 1972). Lindsley and co-workers (1967) have found that stimulation and ablation of the subthalamic region, including the ZI, alter the responsiveness of the midbrain tegmentum to peripheral influxes. Further investigation of the effect of lesions of the ZI on other reflex activities of the rat should be carried out to

determine the extent of this influence.

Sensory and motor functions have been attributed to several structures immediately adjacent to the ZI but it is unlikely that the effects observed in the present study were due to damage to these. The major projections of the dorsal column nuclei (Lund and Webster, 1967) and trigeminal nucleus (Smith, 1973) ascend dorsal to the ZI, but lesions of this region did not reduce water intake. Some lesions partially disrupted pallidofugal fibers of the H_1 field of Forel but damage to this region was not observed consistently enough to explain the experimental results, nor did dorsal lesions causing more extensive damage to this bundle produce the hypodipsia. ZI lesions in the present study were medial to the nigrostriatal pathway as described by Jacobowitz and Palkovits (1974) and medial and dorsal to the subthalamic nucleus.

Motivational Deficits

Undoubtedly, processes of sensorimotor integration and motivation are intimately related and may be difficult to separate at a behavioral level. Although the emphasis of this discussion has been on sensorimotor aspects of the impairment in water intake after lesions of the ZI, the possible contribution of motivational deficits should not be ignored. This is especially relevant in light of the demonstration of sensory inputs to the ZI, since the

reinforcing properties of oropharyngeal activity and feedback in the act of ingestion have been emphasized by several authors (Epstein, 1967; Kissileff, 1973; Oatley, 1973). When oral feedback is reduced (trigeminal deafferentation, Zeigler, 1973) or the oral route of ingestion bypassed altogether by requiring rats to bar press to inject fluids directly, either intragastrically or intravenously (Snowden, 1969; Kissileff, 1973; Nicolaïdis and Rowland, 1974), there appears to be a concomitant reduction in the motivation to feed or drink. Although rats will bar press to maintain minimum water balance through intragastric or intravenous infusions of water, they are deficient in their response to stimuli which readily elicit oral intake of water (Kissileff, 1973; Nicolaïdis and Rowland, 1974; Rowland and Nicolaïdis, 1974). Snowden (1969) reports that rats required to bar press to obtain intragastric injections of food often engage in vigorous licking and chewing movements when feeding in this way, further suggesting that oropharyngeal activity is an integral part of the rewarding properties of ingestion.

Thus, it is possible that motivational deficits accompany the oropharyngeal changes after lesions of the ZI and may contribute to the general suppression of ad libitum water intake. Such a deficit may be involved in the failure of lesioned rats to ingest as much quinine solution as controls, even though the volume obtained per

lap was similar (Section 3.6), and in the transient adipsia observed after lesions were made through chronically-implanted electrodes (Section 3.4). Neural elements of the ZI may be involved not only in the integration or facilitation of oropharyngeal sensorimotor mechanisms but also in the activation of central reinforcement processes associated with the act of ingestion; connections of the ZI with limbic forebrain and midbrain structures may be important in this regard (Section 1.3).

Implications for the Study of Neural Substrates Underlying Motivated Behavior

The results of the present study should be considered in the context of several other recent reports relating the effects of diencephalic lesions to sensory and motor dysfunction rather than to specific motivational deficits in hunger and thirst. A few early reports (Morgane, 1961a; 1961b; Baillie and Morrison, 1963) raised the question of the contribution of motor impairment to the disruptive effects of hypothalamic ablation on ingestive behavior, although these had little impact in the general trend towards motivational interpretations. The demonstration of ascending catecholamine fibers of passage through this region of the brain and the reports that selective damage to these produce many of the symptoms of hypothalamic ablation has reopened this issue, particularly

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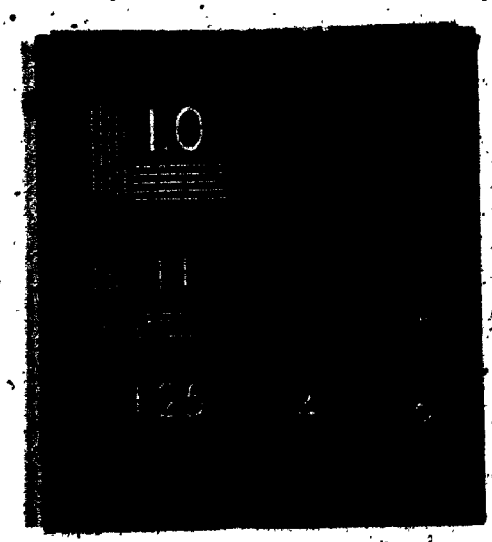

since it is unlikely that these fibers are specifically involved in ingestive behavior (Ungerstedt, 1971b; Marshall and Teitelbaum, 1973; Marshall et al., 1974; Mogenson and Phillips, 1975). Ungerstedt has summarized the problem quite well in reference to the nature of the damage to the ascending nigrostriatal pathway:

> "When considering the curious hypokinesia, lack of exploratory behavior and difficulty to initiate activity that occurs after selective lesions of the nigrostriatal system it is more probable that the DA [dopamine] system and the striatum control a general arousal or drive level that is necessary for performing a number of activities where *eating and drinking deficits are noticed only because they are easily measured by the observer and disastrous to the animal.*" (Ungerstedt, 1971b, p. 116, my italics)

Several other recent studies have demonstrated the presence of sensory neglect (Marshall et al., 1971) and sensorimotor dysfunction (Turner, 1973) after lesions within the lateral hypothalamus (LH). More than damage to the ascending catecholamine pathways is likely involved in these deficits, since Zeigler and Karten (1974) have found that disruption of second order trigeminal fibers which pass through this region of the brain also produce many of the symptoms of LH ablation. Finally, it is also important to consider the effects of damage to descending hypothalamic influences on general motor activity (Wayner, 1975). Thus, it has become increasingly uncertain to what extent the effects of ablation in this

region of the brain can be attributed to specific motivational deficits.

The demonstration of sensory and motor deficits after lesions in the ventral diencephalon, and particularly the subtle oropharyngeal impairment observed in the present study after lesions restricted to the ZI, emphasizes the caution that is required in interpreting the effects of brain lesions on behavior. An informative example is the deficit in the drinking response to cellular dehydration that is observed in rats with lesions of the ZI when solute loads comparable to those usually reported in the literature are injected (Section 3.5). Lesions of several regions of the brain have been noted to attenuate drinking in response to injections of hypertonic solutions and the results have been interpreted as demonstrating the presence of osmosensitive regions or neural pathways mediating the drinking response to cellular dehydration (Blass and Epstein, 1971; Peck and Novin, 1971; Emmers and Passamonte, 1972; Walsh and Grossman, 1974; Kucharczyk and Mogenson, 1975a). Support for such specificity is commonly drawn from the observation that these lesions do not disrupt drinking to extracellular dehydration. However, since drinking in response to injections of hypertonic solutions is particularly sensitive to inhibitory influences (Burke et al., 1972), lesions which produce a slight but non-specific suppression of water intake as observed in the present study may also

cause a selective attenuation of drinking to cellular dehydration. Therefore, a more cautious interpretation of results is in order until non-specific effects can be excluded.

In this regard, the results of the present study demonstrate that the standard techniques, as they are normally used for testing regulatory mechanisms, may not be adequate for characterizing post-lesion deficits. Not only may apparently specific effects of lesions be caused by non-specific impairments as described above, but the failure of ablations to attenuate the drinking response to primary cues does not conclusively demonstrate that these responses are unaffected, as shown in Section 3.5. Thus, more careful interpretations based on a better understanding of the behavioral and physiological characteristics of the regulatory processes themselves are needed.

The demonstration of sensorimotor impairments after lesioning may also have important implications for the interpretation of stimulation studies. The finding that lesions of the ZI reduce daily water intake is consistent with the observation that electrical and chemical stimulation of this region elicits drinking behavior (Section 1.3), though it cannot be certain that the same neural elements are being affected. Although it is often assumed that electrical stimulation elicits drinking by ..

activating specific neural circuits involved in the regulation of water intake (Mogenson, 1973), other alternatives have been proposed. One possibility, recently advocated by Wayner (1975), is that stimulation may elicit ingestive behaviors through the facilitation of sensorimotor systems associated with oropharyngeal activity. It has been reported for the cat (MacDonnell and Flynn, 1966) and rat (Smith, 1972) that hypothalamic stimulation causes the appearance of sensory fields in the perioral region for head orienting, jaw opening and biting. Noting the convergence in the ventral diencephalon of afferents and efferents involved in oropharyngeal control, Wayner (1975) points out that stimulation in this region may result in the simultaneous facilitation of muscle movements and related sensory feedback involved in the control of these movements. Therefore, consideration should be given to the possibility that drinking behavior elicited by electrical stimulation of the ZI is at least partly due to the facilitation of oropharyngeal sensorimotor systems involved in licking and swallowing fluids.

Perhaps a similar mechanism is involved in the elicitation of drinking by cholinergic stimulation. Acetylcholine esterase-containing cell-bodies have been observed in the ZI (Jacobowitz and Palkovits, 1974) and cholinergic stimulation of the ZI, like many other structures, elicits water intake in the rat (Hulst,

1972). It is noteworthy that the effects of cholinergic blockade on drinking behavior are quite similar to those of lesions of the ZI. First of all, the peripheral (Blass and Chapman, 1971) or central (Fisher, 1973) application of atropine or scopolamine only partially reduces drinking following intracellular or extracellular dehydration, although there are some reports (De Wied, 1966; Block and Fisher, 1970) that anticholinergic drugs attenuate the drinking induced by injections of hypertonic solutions (see above discussion of the specificity issue). Secondly, the effects of atropine on drinking following various periods of water deprivation are similar to those presented in Figure 4 for rats with lesions of the ZI; the decrease in intake does not vary with the length of deprivation (Block and Fisher, 1970; Krikstone and Levitt, 1970). Finally, lesions of the LH which extend into the ZI have been reported to block the drinking response to cholinergic stimulation of limbic sites (Wolf and Miller, 1964; Stein and Levitt, 1971). Like the effect of electrical stimulation, it has generally been assumed that chemical stimulation activates neural elements involved in the regulation of water intake and the similarity between cholinergic activation and natural thirst has been emphasized (Fisher, 1973). However, in light of the results observed for ZI lesions, it may be relevant to consider

the effects of cholinergic stimulation and blockade on general oropharyngeal activity. Furthermore, the direct application of anticholinergic drugs to the ZI may also be useful in further investigating the function of this region of the brain.

Finally, it is relevant to re-examine one of the earlier findings of this study, that lesions of the ZI attenuated secondary drinking. As evident in Chapter 1, the major emphasis in the study of the control of water intake has been on the role of regulatory drinking responses to body water deficits, and on the investigation of neural substrates underlying these regulatory responses. It is significant, therefore, that lesions of the ZI reduced daily water intake without attenuating drinking to intracellular or extracellular dehydration. Animals do not drink solely in response to body water deficits but also under many conditions when there is no apparent need for water to maintain body fluid homeostasis (Sections 1.1, 3.3), categorized by Fitzsimons (1971) as secondary drinking. There has been increasing recognition of the importance of secondary drinking in ad libitum water intake and it has been proposed that it is secondary influences related to endogenous rhythms, environmental constraints, and learned associations that actually govern the amount and pattern of daily water intake (Fitzsimons, 1972; Hirsch and Collier,

1974). The significance of these secondary controls is that they may actually anticipate water requirements, enabling animals to ingest water before a deficit occurs. In this way, under stable environmental conditions, animals may set up patterns of water intake appropriate to their ecological niche and behavioral priorities and not solely at the command of internal physiological needs.

Although it is obvious that animals "do not drink reflexly, like automatons to the adequate physiological stimuli" (Epstein, 1973, p. 320), relatively little attention has been directed towards determining the underlying neural substrates for secondary drinking. The present study has been somewhat unique in this respect, although the results of subsequent experiments did not support a hypothesis that neural elements of the ZI play a specific role in the control of secondary water intake. Nevertheless, this should not detract from the importance of considering the possibility that lesions and stimulation of other structures influence secondary drinking mechanisms. Secondary controls of water intake undoubtedly involve elements of learning, endogenous rhythms and neural programming related to the so-called "higher" functions of the central nervous system and hence may provide a valuable model for the investigation of these activities. In this respect, the need for

further behavioral and physiological investigation of secondary phenomena and their relation to the more classical theories of physiological regulation is evident.

4.2 Summary

1. The results of several recent studies have suggested that neural elements of the zona incerta (ZI) participate in the control of drinking behavior in the rat. To investigate the role of the ZI in the control of water intake, experiments were conducted to characterize the deficit in drinking behavior after electrolytic lesions in this region of the brain.

2. A total of 217 male Wistar rats was used in the study, in 9 series of 20-30 rats each. Animals were assigned to groups for data analysis solely on the basis of histological determination of site and extent of the lesions. The effects of bilateral lesions of the ZI (78 rats) were compared with the effects of sham lesions (60 rats) and of control lesions dorsal to the ZI (32 rats).

3. Bilateral lesions of the ZI, but not sham lesions or lesions dorsal to the ZI, produced a 20-30% reduction in daily water intake in rats with no concomitant changes in food intake or body weight. This hypodipsia showed no sign of recovery up to 2 months after surgery and was characterized by a level of water intake (16-18 ml) that varied little between rats.

4. When the fluid intakes of lesioned and control rats were matched by maintaining them on a dilute liquid diet no

significant difference was found in the daily volume or osmolality of urine or Na^+ and K^+ excretion. Lesioned and control rats excreted urine at similar rates following intragastric water loading. It was concluded that the reduced water intake after lesions of the ZI was due to a direct effect on mechanisms controlling water intake and not to alterations in daily water requirements or excretory mechanisms.

5. Compensatory reductions in water losses accompanied the reduced water intake; a 30% reduction in urine volume and increase in urine osmolality were observed in lesioned rats when water was available ad libitum. There were no significant differences between lesioned and control rats in hematocrit, serum osmolality or Na^+ and K^+ levels. Neither were any differences in rectal temperature observed.

6. Lesions of the ZI caused only a transient (2-3 wk) reduction in spontaneous running-wheel activity which was not significantly correlated with the reduction in water intake. It was concluded that the hypodipsia was not due to a non-specific depression of behavior.

7. Rats with lesions of the ZI drank as much as controls in response to intracellular dehydration induced by ip injections of hypertonic saline, and extracellular dehydration induced by sc injections of hyperoncotic colloid. Following water deprivation (6-48 hr)

lesioned rats drank 2-3 ml less than controls, but this difference in intake did not vary with length of deprivation. It was concluded that the reduction in ad libitum water intake was not due to failure to respond to signals of water deficit.

8. Normally animals do not drink solely in response to body fluid deficits but also under conditions when no need for water to maintain fluid homeostasis exists, termed secondary drinking. The effect of lesions of the ZI on drinking occurring in excess of that required to maintain water balance was determined. Rats were maintained on a dilute liquid diet (73% water) designed to meet daily water requirements. Before lesioning, an additional 5-10 ml of water was ingested, but lesions of the ZI abolished this extra drinking.

9. In the absence of secondary drinking water intake should be more precisely regulated to actual water requirements for fluid homeostasis. When daily water requirements were altered by varying diet protein content (from 20-70%), the daily water intake of rats with lesions of the ZI but not control animals closely followed the changes in fluid need. It was concluded that ZI lesions reduce ad libitum water intake in rats by attenuating secondary drinking.

10. Since secondary factors have been implicated in the control of daily patterns of ingestion, food jars and

water spouts were equipped with devices to continuously record feeding and drinking activity. Before lesioning, a circadian rhythm and close temporal relationship between feeding and drinking were observed; 70-80% of daily food and water intake were ingested at night, and 70-80% of drinking occurred within 10 min before or after a meal.

11. Lesions of the ZI, made through implanted electrodes in conscious rats, produced a temporary disruption (4-6 hr) of the normal pattern of ingestion. Following an initial burst of alternate feeding and drinking (1/2 hr), a transient adipsia was observed; 3-4 meals occurred unaccompanied by drinking.

12. Except for the initial disruption of feeding and drinking, lesions of the ZI did not disrupt normal patterns of ingestion. Lesioned rats did not engage in the prandial style of drinking reported for rats with lesions of the lateral hypothalamus; less than 30% of the meals were interrupted by drinking and the mean size of drinks occurring during meals was greater than 0.5 ml.

13. The reduction in total water intake of lesioned rats was due to the ingestion of smaller but not fewer drinks. Furthermore, lesioned rats lapped as frequently at the water spout as control animals (3500-4000laps/day) but obtained 15-20% less water per lap, suggesting an

impairment in the ability to ingest water.

14. Rats with lesions of the ZI drank less than controls after water deprivation when the drinking solution was made bitter by adding quinine (0.0001-0.1 gm/100 ml) or sweetened with saccharin (0.001-1.0 gm/100 ml) and the fluid intake of lesioned rats was reduced by lower concentrations of quinine than that of controls. These results demonstrated a subtle but significant deficit in the drinking response to dehydration as well as secondary drinking and supported a hypothesis of a more general inhibition of drinking after lesions of the ZI.

15. When the drinking response to cellular dehydration was re-examined, it was observed that rats with lesions of the ZI drank as much water as controls after the injection of low (0.5M NaCl, 4 ml/kg) but not higher (2M NaCl, 2 ml/kg) solute loads. The results are discussed in terms of a non-specific inhibition of water intake after lesioning.

16. Experiments were conducted to investigate whether lesioned rats were impaired in the ability to ingest fluids (see 13). Rate of licking was not different in lesioned and control rats (5-6 laps/sec), but lesioned rats obtained 30% less water per lap at the drinking spout than controls after 12 or 24 hr water deprivation and 55% less liquid diet per lap after 24 hr

water or food deprivation. Lap volume varied with palatability of the drinking solution in control rats (4-8 µl) but not in rats with lesions of the ZI (4-5 µl).

17. In conclusion, the results of the experiments demonstrate that electrolytic lesions of the ZI reduce ad libitum water intake in rats by attenuating secondary drinking and restricting daily water intake to minimal requirements for water balance. This reduction in intake is not due to a specific disruption of neural elements involved in the control of secondary drinking but to a more general inhibition on water intake, related to an impairment in the ability of lesioned rats to ingest fluids. These results are discussed in terms of neuroanatomical connections of the ZI and the role of this region of the brain in oropharyngeal control. The implications of these findings for the investigation of neural substrates involved in motivated behavior are also considered.

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