ANTERIOR NEOCORTICAL KINDLING

IN VASOPRESSIN-DEFICIENT BRATTLEBORO RATS

by

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ABSTRACT

Several lines of evidence have suggested that vasopressin present in the central nervous system may influence the development of seizures.

Homozygous and heterozygous Brattleboro rats deficient in central vasopressin and Long-Evans control rats were electrically kindled in the anterior neocortex.

There were no consistent group differences in afterdischarge threshold, the rate of kindling, or percentage of refractory sessions. However, Brattleboro groups had longer afterdischarge durations prior to generalization.

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Table of Contents

Table of Contents (cont.)

Tables

Figures

Key to Abbreviations

AD = afterdischarge

ADD = afterdischarge duration

ADH = antidiuretic hormone

AVP = arginine vasopressin

HE = heterozygous Brattleboro rats

HO = homozygous Brattleboro rats

GC = generalized convulsion

i.c. = intracarotid

i.c.v. = intracerebroventricular

i.p. = intraperitoneal

i.v. = intravenous

LVP = lysine vasopressin

µA = microamps

PV = paraventricular hypothalamic nucleus

SC = suprachiasmatic hypothalamic nucleus

s.c. = subcutaneous

S.D. = standard deviation

SE = standard error

SO = supraoptic hypothalamic nucleus

VP = vasopressin

Tables: - = lower than normal
0 = no change from normal
+ = higher than normal

Anterior Neocortical Kindling in Vasopressin-Deficient Brattleboro Rats

The mammalian hormone vasopressin (VP) has provided decades of experimental evidence regarding its classical function as the principal antidiuretic hormone (ADH).[1] VP, like its structural analogue oxytocin, is a nonapeptide released from the posterior pituitary (neurohypophysis). Most mammals have the form known as arginine vasopressin (AVP), in reference to the eighth amino acid in the peptide, arginine. The pig has an identical VP except that lysine replaces arginine (LVP).

VP acts to limit water excretion and to constrict vascular smooth muscle. Such antidiuretic and vasoconstrictive properties normally help to maintain proper blood pressure and osmolarity. Baroreceptors, osmoreceptors, chemoreceptors and possibly thermoreceptors located on or near neurons of the supraoptic (SO) and paraventricular (PV) hypothalamus regulate release of VP. Release into the peripheral blood (neurosecretion) takes place from primary or secondary axons terminating in the neurohypophysis. Conditions requiring release of VP include the normal maintenance of blood pressure and osmolarity but also such emergent conditions as hemorrhage, stress and hypoxia (Rydin & Verney, 1938; Vorherr, Bradbury, Hoghoughi,& Kleeman, 1968;

Schrier, Berl & Anderson, 1979; Wang, Share, Crofton & Kimura, 1981).

Lesser known evidence suggests that AVP may also be involved in a variety of functions both peripherally and in the central nervous system (CNS). AVP is found not only in the blood and neurosecretory pathways of hypothalamic nuclei, but also throughout the CNS. It has been known for decades that the cerebrospinal fluid (CSF) contains an antidiuretic substance more recently identified as AVP (Cushing & Goetsch, 1910; Dogterom, van Wimersma Greidanus & de Weid, 1978; Jenkins, Mather & Ang, 1980; Vorherr, et al., 1968; Wang, Share, Crofton & Kimura, 1981; Zaidi & Heller, 1974). AVP is also found throughout the brain itself (Buijs, 1978; Hawthorn, Ang & Jenkins, 1980; Sofroniew & Weindl, 1978).

Immunohistochemical evidence suggests that extrahypothalamic brain AVP is contained within distal axons of neurons whose cell bodies lie in the suprachiasmatic (SC) and PV hypothalamus. The SC nucleus has been found to provide fibers that make axo-somatic contacts with neurons in projection areas (Sofroniew & Weindl, 1978). Table 1 reviews findings from immunohistochemical, histochemical and radioimmunoassay studies on the projection area distributions of brain AVP. More than one laboratory has confirmed the presence of extrahypothalamic AVP in the septum, thalamus, medulla, amygdala, substantia nigra, hippocampus, pons and

Table 1
Immunohistochemical (I), Radioimmunoassay (R) and Histochemical (H) evidence of AVP or its carrier protein, neurophysin II in the brain excluding hypothalamo-hypophyseal pathways.

Authors	Date	Method	Species	Brain region AVP or Neurophysin II found
Robinson & Zimmerman	'73	H	Rat	Neurophysin II in ependymal tanycytes of the third vent
Vandesande, DeMey & Dierickx	'74	I	Rat	Suprachiasmatic nucleus of the hypothalamus.
George & Jacobowitz	'75	I	Rat	None described outside the hypothalamus.
Weindl & Sofroniew	'76	I	Guinea Pig	Neurophysin II and AVP fibers found outside of the hypothal.
Sofroniew & Weindl	'78	I	Rat	Small neurophysin II fibers from parvocellular SCN to septum and dorsal thalamus.
Sumy-Long, Keil & Severs	'78	I	Rat	AVP in subfornical organ, fornix and ant. commissure.
Dogterom,	'78	R	Rat	AVP found in the septum, Snijdewint organum vasculosum lamina & Buijs terminalis, amygdala, hippocampus, cortex, choroid plexus, medulla oblongata.
Buijs, Dogterom	'78	I	Rat	AVP fibers found in the striae Swaab, terminalis and lateralis and neurosecretory fibers under ventricular ependyma
Buijs	'78	R	Rat	AVP fibers from PV nucleus to he hippocampus, amygdala, substantia nigra, substantia grisea, nucleus solitarius, nucleus ambiguous, substantia gelatinosa. Also fibers from the SCN to lateral habenula.
Hawthorn Ang & Jenkins	'80	R	Rat	AVP found in hypothalamus,thalamus, cerebellum, medulla, amygdala, substantia nigra, hippocampus, pons, spinal cord, neocortex.

spinal cord (Buijs, 1978; Hawthorn et al., 1980; Sofroniew & Weindl, 1978).

The levels of brain AVP have been quantified in rats using radioimmunoassay methods. Concentrations vary from a high of 2066pg/mg protein in the hypothalamus with lower levels down to 30 pg/mg found in the amygdala, substantia nigra, cerebellum and thalamus. Amounts of from 27 to 5.7 pg/mg are found in the hippocampus, pons, spinal cord, occipital lobe, caudate-putamen and frontal lobe in descending concentration (Hawthorn et al., 1980).

It is worthy of note that AVP levels in the CSF vary according to a circadian cycle whereas AVP levels in the blood vary according to osmotic factors. These and other findings suggest that the role of AVP in the CNS may be different than that in the periphery (Reppert, Artman, Swaminathan, & Fisher, 1981; Reppert, Schwartz & Uhl, 1987).

A Possible Neurotransmitter or Neuromodulatory Role of AVP:

Since AVP is released from axons ending in the posterior pituitary, it is plausible that AVP may also be released from other axons in which AVP has been demonstrated. Various studies have suggested a functional role for AVP released within the CNS. These studies concern influences on learning and memory (De Weid & Bohus, 1966; De Weid, 1976), temperature regulation (see below), cardiovascular regulation

(Matsuguchi, Sharabi, Gordon Johnsson & Schmid, 1982; Pittman, Lawrence & McLean, 1982; Pittman & Franklin, 1985), and spontanous motor behavior (Kruse, Van Wimersma Greidanus & DeWeid, 1977; Ferris, Albers, Wesolowski, Goldman & Luman, 1984). In summary, the evidence suggests that (a) AVP is contained in--and may be released from--axons within the CNS and (b) AVP injected into various brain foci has behavioral and physiological effects. These findings suggest a role for AVP which is most readily conceptualized according to that of a neuromodulator or neurotransmitter. Neurophysiological studies using a variety of methods have provided more direct evidence.

A neuromodulatory role requires that AVP is released by neurons and thereby alters neuronal function in a demonstrable manner. Studies in rats have shown that the response of lateral septal neurons to activation of afferent pathways or to infusions of excitatory neurotransmitters is enhanced by coincident vasopressin infusions (Joels & Urban, 1984). If in fact AVP is released by neurons, it is likely to have at least a neuromodulatory role.

Evidence for a possible neurotransmitter role has emphasized the presence of AVP receptors in the CNS. Autoradiographic localization of [^{3}H]AVP binding sites in the brain provide direct evidence of AVP receptors in the CNS (Van Leeuwen & Wolters, 1983). Binding was demonstrated

for the cortex and medullary regions and was particularly intense in the lateral septum. The lateral septum has recently been shown to receive AVP-containing projections from the medial amygdaloid nucleus (Caffe, Van Leeuwen & Luiten, 1987), in addition to previously known AVP-containing fibers from the hypothalamus. These findings suggest that AVP receptors are concentrated in regions in which AVP-containing fibers terminate, which is consistent with a neurotransmitter role.

Further evidence for a neurotransmitter role includes the finding that synthetic AVP agonists and AVP antagonists may act selectively on CNS--as opposed to peripheral--receptors for AVP. Using passive avoidance latency as a measure of central effects and heart rate and blood pressure to measure vasopressor effects, De Weid, Gaffori, Van Ree and De Jong (1984) distinguished specificities between two synthetic analogs of AVP. They found a high level of potency of one agonist (AVP4-8) on central receptors but essentially no potency of the same agonist on peripheral receptors. A more recent study by Burnard, Veale and Pittman (1986) provides evidence that motor disturbances elicited by intracerebroventricular injections of AVP are receptor mediated. Pretreatment with an AVP receptor antagonist prevented ~~motor~~ "convulsive-like" motor disturbances resulting from AVP infusions but had no affect on the effect

of other convulsants. Taken together, these and other studies suggest that the CNS functions of AVP are receptor mediated and that these receptors are CNS-specific. The presence of a unique CNS AVP receptor is substantiated further by studies showing altered potency and dissociation constants of ligands in various bioassays and binding studies (Schriffin & Genest, 1983; Tiberiis, McLennan & Wilson, 1983; Lawrence, Poulin & Lederis, 1984).

Finally, a neurotransmitter role has been supported by several studies on the effect of AVP infusions on neuronal activity. In a study by Muhlethaler and Dreifuss (1982), infusions had an excitatory effect on rat hippocampal neurons in brain slice preparations. A vasopressin receptor antagonist reversed this effect. It is also interesting to note that dose-response relationships for AVP did not differ between homozygous Brattleboro rats and their Long-Evans parent strain, suggesting the absence of AVP receptor abnormalities in the Brattleboro rat. Other studies have reported direct excitatory or inhibitory effects of AVP infusions on neuronal activity in the septum and dorsal hippocampus (Joels & Urban, 1982), supraoptic nucleus (Nicoll & Barker, 1971) and locus coeruleus (Olpe & Baltzer, 1981).

The AVP Gene and Diabetes Insipidus:

DNA for the AVP gene has been sequenced in the rat (Schmale, Heinsohn & Richter, 1983; Furutani, Mormoto,

Shibahara, Noda, Takahashi, Hirose, Asai, Inayama, Hayashida, Miyata & Numa, 1983) and in the calf (Ruppert, Scherer & Schutz, 1984). For these and other mammals, the amino acid sequence for AVP and oxytocin differ in only two out of nine amino acids (positions two and eight). All but six out of 27 nucleotides coding for these peptides are homologous. Consequently, when comparing vasopressin and oxytocin, the homology of either the nucleotide sequence or their amino acid transcripts is 78%.

The gene for AVP is contained on one exon (Exon A), which contains in sequence: a signal peptide, the AVP gene, an untranslated sequence of three bases, and a small segment of the neurophysin II gene. The balance of neurophysin II is coded on exons B and C. Exons B and C are separated from Exon A--and from each other--by introns, that is, sequences of DNA which are not translated into protein (Ivell & Richter, 1984).

The mutation responsible for diabetes insipidus in the Brattleboro rat involves the deletion of a single guanosine base. This mutation lies in the Exon B portion of the neurophysin II gene. The deletion gives rise to an open reading frame with an altered C-terminus (Schmale & Richter, 1984). It is curious that the mutation responsible for the AVP deficiency involves AVP only indirectly, via its carrier protein.

Circumstantial Evidence for the Role of AVP in Thermoregulation:

The focus of this paper is the role of AVP in the production of seizures. Research regarding this role is grounded in studies of other functions, most notably temperature regulation. A review of these and other findings will demonstrate that the various potential functions of AVP are not wholly independent (see for instance Lee & Lomax, 1983). In fact, it was through the investigation of the thermoregulatory effects of intracerebroventricularly (i.c.v.) administered AVP that workers at the University of Calgary discovered what appeared to be convulsant effects (Kasting, Veale & Cooper, 1980). This led to the hypothesis that AVP might play a role in the etiology of febrile seizures (Kasting, Veale, Cooper & Lederis, 1981). Consequently, the role of AVP in thermoregulation will be reviewed first, followed by studies on the role of AVP in febrile and non-febrile seizures.

An immunity to fever from infectious agents has been reported for newborn mammals including guinea pigs (Blatteis, 1975), sheep (Pittman, Cooper, Veale & Van Petten, 1973) and humans (Bergstrom, Larson, Lincolm & Winberg, 1972; Epstein, Hochwald & Ashe, 1951; Smith, Platou & Good, 1956).

In the early investigations of fever immunity in lambs and ewes, Calgary workers noted that a different laboratory

had shown an increase in plasma levels of AVP in the ewe and her fetus near parturition (Alexander, Britton, Bashore & Forsling, 1974; Cooper, Kasting, Lederis & Veale, 1979; Kasting, Cooper & Veale, 1979) Such changes in AVP levels corresponded to the period in which ewes would not develop a fever as normally seen after injections of bacterial endotoxin.

Other studies had pointed more directly to a role for AVP in temperature regulation. The earliest of these studies showed that levels of plasma AVP, LVP or ADH activity were seen to increase in the following hyperthermic conditions: (1) in men working in hot and humid environments (Weiner, 1944; Segar & Moore, 1968); (2) in rats exposed to high ambient temperatures (Itoh, 1954); (3) in dogs when the preoptic area was heated to 1.5 C above normal (Szczepanska-Sadowska, 1974); (4) in pigs in which the mean rectal temperature increased from 39 to 43 C during ambient heating (Forsling, Ingram & Stanier, 1976); (5) in rabbits with injections of bacterial (Escheria Coli) pyrogens (Kruk & Sadowski, 1978) and (6) in sheep with bacterial (Salmonella abortus equi) pyrogen-induced fevers (Cooper et al., 1979). In the latter study, increased levels of AVP were also found in the septal area of sheep in association with fever. (See Table 2.)

The observation that endogenous levels of AVP correspond

Table 2.

Effects of imposed temperature changes on endogenous AVP.

Authors	Date	Species	Method of Heating	Site of VP (or ADH) assay	Effect on VP (or ADH conc.
Itoh	'54	Rat	whole body heating	plasma ADH	+
Okuno Yamamoto & Itoh	'65	Rat	whole body heating	plasma ADH	+
Szczepan-ska-Sad-owska	'74	Dog	localized to basal forebrain	plasma VP	+
Forsling, Ingram & Stanier	'76	Pig	whole body heating	plasma VP	+

Key to abbreviations in Tables 1-4:

Under "Authors":

"C" = Cooper
"E" = Eagan
"K" = Kasting
"L" = Lederis
"V" = Veale

to conditions of elevated body temperature in no way distinguishes what that role might be. It is worth pointing out that blood AVP increases substantially as a result of emotional stress alone (Rydin & Verney, 1938; Schrier et al., 1979). If AVP is released as a nonspecific consequence of emotional stress, it might be expected that prolonged exposure to heat, intracranial heating or intracranial cannulae, could, along with the handling necessary to measure AVP levels, induce emotional stress in experimental animals. Whereas many of such experiments listed above utilized unheated or non-injected (pyrogen-free) animals, such controls do not remove the possibility that the experimental condition involving pyrogens, or even heat itself, could produce nonspecific stress unique to the experimental group. Hence, it was proper that Cooper and coworkers observed that elevation of AVP in either the blood or brain during fever "is an interesting association but, by itself, suggests neither cause nor effect" (Kasting et al., 1979, p. 41).

Evidence for a functional role of AVP release in heat reduction:

Several studies have attempted to determine if AVP could influence fever or hyperthermia from heat exposure (Table 3). Cooper and coworkers (1979) infused approximately 6.4

Table 3.

Effect of AVP injections on fever or artificial heating.

Authors	Date	Species site	inj dose AVP	AVP site	inj dosage endo	endotoxin	effect on fever °C
K,C & V (or C,K,L & V)	'79	Sheep	Sept	6.4µg	i.v	30µg	reduced by .75 C
C,K,L & V	'79	Sheep	i.v	24µg	i.v.	30µg	unchanged
V,E & C.	'82	Ho-Rat	s.c.	2.4mg	i.c.v		.1µg increased by 1.2 C
Okuno, Yamamoto & Forsling	'65	Rat	s.c.	15µg 100gm	ambient heating		no effect

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micrograms (μg) of AVP into the septal nucleus of normal adult sheep over the first 200 minutes following peripheral injection of endotoxin. The resulting fever was markedly reduced. Peripheral injections of AVP were ineffective, which is consistent with findings by Okuno et al. (1965), who showed that subcutaneous injections of AVP do not reduce temperature elevation resulting from ambient heating. Finally, Cooper and coworkers also showedthat central injections of AVP had no effect on resting temperature.

Table 4 reviews studies of various AVP injections and resting temperature. The inability of intraseptal injections of AVP to affect resting temperature contrasted remarkably with results of peripheral or intracerebro-ventricular (i.c.v.) injections. Okuno and coworkers (1965) have shown that AVP reduces resting temperature in rats when injected (1) subcutaneously (s.c.) at a dosage of 200-300μg/100g body weight; (2) intraperitoneally (i.p.) at 8-12μg/100g; (3) intracarotidallyi.c.) at 4-6μg/100g; or (4) intravenously (i.v.) at 4-6μg/100g.[2] More recent results suggest that while s.c. injections (115-230μg/100g) of AVP could reduce resting temperature of rats 100% deficient in AVP (Veale, Eagan & Cooper, 1982), i.c.v. infusions (0.2-0.4μg/100g) were also effective in reducing resting temperature in normal rats.

Table 4.

Effect of AVP injections on resting temperature.

Authors	Date	Species	injection site AVP	AVP dose	effect on resting temperature
Okuno, Yamamoto & Itoh	'65	Rat	i.p. i.v.		- -
Kruse, Van Wimersma Greidanus, de Weid	'77	Rat	i.c.v.	200ng	-
C,K,L & V	'79	Sheep	Sept	6.4µg	0
V,E & C	'82	Ho Brat	s.c.	1/2.4mg	-
K,V & C	'80	Rat	i.c.v.		-

Under "Authors":

"C" = Cooper
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In summary, it seems that AVP acts to reduce resting temperature when injected peripherally or i.c.v., but not when injected in small amounts into the septal nucleus of comparably large-brained mammals. In contrast, AVP administered peripherally fails to affect elevated body temperatures resulting from either endotoxin-induced fever or from induced hyperthermia, whereas intraseptal infusions reduce fever significantly. Consequently, dual actions have been proposed for the suppression of resting temperature by peripheral AVP (Okuno et al., 1965) and the suppression of fever by septal AVP (Cooper et al., 1979).

Blood-CSF and Blood-Brain Barriers to AVP:

Numerous studies using a variety of methods have shown that AVP does not pass the blood-CSF barrier (Jenkins et al., 1980; Vorherr et al., 1968; Wang et al., 1981; Zaidi & Heller, 1974). However, AVP may pass the blood-brain barrier, which is distinguished from the blood-CSF barrier (Minckler, 1972). Hypothalamic cell bodies giving rise to AVP-containing axons are in close proximity to known gaps in the blood-brain barrier. These regions include the median eminence, the subfornical organ, and the organum vasculosum lamina terminalis. These areas make up part of the ventral zone of the rostral hypothalamus, ventral to AVP-containing neurons in the hypothalamus. It may be concluded that while

AVP does not enter the CSF from the blood, it may enter the hypothalamus from the blood. This was the apparent suggestion of Kasting and coworkers, who wrote "Although blood levels do not necessarily indicate what may be occurring in the brain, they may nonetheless, reflect massive release of AVP directly into the brain itself or into the blood in sufficient quantities to penetrate the brain." (Kasting et al., 1981, p. 331).

These conclusions are also supported by behavioral studies serving to compare the effects of intracranial and peripheral injections of AVP. Either route is effective in increasing the retention of avoidance responses in rats. Peripheral injections required much greater doses (0.54 μg) than intracranial injections (2.5ng), but this is to be expected since the injected amount is spread throughout the body (de Weid, 1976).

Evidence for AVP Release in Fever Production: Peripheral vs. Central Roles

Whereas the aforementioned studies suggest an antipyretic role for central (intraseptal) AVP, other studies by the same workers suggest just the opposite for peripheral AVP. These findings were reported in studies of Brattleboro rats, of which rats homozygous (HO) for a certain gene are 100% deficient in AVP (Mohring & Mohring, 1975). Injections of bacterial endotoxin failed to produce fever in such rats,

suggesting that the absence--rather than presence--of AVP is antipyretic (Eagan, Kasting, Veale & Cooper, 1982). Further studies suggested the possibility that other abnormalities of Brattleboro rats (see Sokol & Valtin, 1982) were responsible for their lack of fever and that AVP itself was the missing necessary factor: AVP deficient rats received subcutaneous injections of AVP prior to injections of endotoxin. Resting temperature went down as expected when AVP was injected peripherally; however, subsequent injections of endotoxin into the cerebral ventricles reversed this effect, leading to fever. The authors concluded that in response to endotoxins "when AVP is present fever occurs and when it is absent it does not," (Veale et al., 1982 p.777). They suggested that AVP was required to produce endogenous pyrogen in reticulo-endothelial system subsequent to bacterial endotoxin.

One might conclude that while AVP may act as an antipyretic intraseptally, it acts peripherally both in reducing resting temperature and also as a necessary ingredient in endotoxin pyresis. This poses the question as to why AVP is released not only centrally, but peripherally during fever (Cooper et al., 1979). It is interesting to speculate that antipyretic functions of central AVP serve to compensate pyretic effects exerted by its peripheral release during fever. Such a peripheral release of AVP might be necessary for more beneficial purposes. During fever, water

loss through the lungs and skin increases, thereby depleting the blood of water. Secretions of AVP into the blood might serve to retain water in the kidneys under such circumstances. An alternative cause of AVP release might be reductions in central blood pressure due to peripheral dilation during hyperthermic states. Since AVP release under such circumstances might have an undesirable side-effect of increasing the production of endogenous pyrogen, a system might have evolved to compensate for this effect by influencing brain mechanisms of temperature regulation. Whereas this antipyresis takes place only through direct action on neurons in the brain, it would be essential that brain levels of AVP correspond to those in the blood in order for the mechanism to work. Such a model conforms to the findings described in many of the above-mentioned studies and in no way eliminates the possibility that AVP may potentiate the production of seizures, especially those seen during fever.

It remains possible that a correspondence between central and peripheral release of AVP evolved at least partly for reasons other than antipyresis. For instance, perhaps correspondence might be necessary to maintain water balance between blood and brain, as suggested by recent studies (Raichle & Grubb, 1978). In support of correspondence of peripheral and central AVP, both physiological and anatomical

evidence suggests that release of AVP into the blood and CNS is linked. It has been observed that AVP is released into both the CSF and into the blood as a consequence of hemorrhage (Burnard, Pittman & Veale, 1983; Vorherr et al., 1968; Wang et al., 1981) or injections of hypertonic saline (Burnard et al., 1983). Although it appears that peripheral AVP would play an adaptive role during hemorrhage or saline injections, it is unclear what role central AVP might have. This stands in reverse of the situation with fever, in which AVP is released both in the septum as well as into the blood, but for which a function is so far identified only for septal AVP.

In addition to the physiological evidence for a link between peripheral and central AVP release, anatomical evidence suggests that some hypophyseal neurosecretory axons and those within the CNS derive largely from the same bipolar neuron (Buijs, Swaab, Dogterom & van Leeuwen, 1978). This suggests that peripheral release coincides with central release in at least some circumstances.

In summary, the distinction between peripheral and central actions of AVP seems to fall along the following lines: Both central and peripheral AVP may be released with the same stimuli. The blood-CSF barrier is unquestionably present for AVP; however, the efficacy of peripheral AVP in central functions has been shown. This suggests the blood-

brain barrier is not absolute and might therefore be distinguished from the blood-CSF barrier.

The etiology of febrile seizures: A possible role for AVP

Thirty percent of all convulsions[3] in children occur in the presence of fever (Meloff, 1971). A number of different etiologies have been suggested. Wortis (1938) held that developing brain tissue requires more oxygen and was consequently at risk during the higher metabolic rates of fever. Wegman (1939) suggested that the rate of temperature rise is more critical than absolute temperature. These conclusions held sway until a reanalysis of Wegman's data showed that the height of temperature rise was actually more important than the rate of rise (Millichap, 1968). (See Lorin, 1982, p 155 for discussion.) Millichap felt that blood chemistry or immune reactions might play a role. Others have considered inherited factors to influence the incidence of febrile seizures (Livingston, 1972, p.31).

There is some controversy whether febrile seizures increase the risk of later epilepsy (See Wolf, 1979). Large prospective studies have shown that when febrile seizures are short in duration (under three minutes) and are not accompanied by focal neurological symptoms, the likelihood of future epilepsy is hardly greater than that of the normal population. However, when febrile seizures are focal and prolonged, and when electrographic abnormalities are seen

between seizures, risk of nonfebrile seizures increases up to 97% (Livingston, 1972, pp 25-31).

Another large prospective study by Van den Berg (1974) showed that risk of nonfebrile seizures increases three to four times with a single febrile seizure and up to thirty times after four or more febrile seizures. Since the appearance of febrile seizures and nonfebrile seizures may be correlated only through a common predisposing factor, these findings do not prove that febrile seizures cause nonfebrile epilepsy. But they may suggest that febrile seizures may either precipitate seizures in children with underlying epilepsy, or alternatively, that such seizures are at least indicative of increased risk of epilepsy.

Penfield and Erickson (1941) postulated long ago that the difference in cellular permeability and water content in the brain tissue of young children may contribute to the etiology of febrile seizures. Therefore it is interesting that brain AVP may serve to maintain brain water balance as mentioned above (Raichle & Grubb, 1978).

The first experimental correlation between a hyperthermic state, seizures and AVP was shown in a study by Kasting and coworkers (1981). They demonstrated that AVP-deficient Brattleboro rats and rats pretreated with anti-AVP serum convulsed at significantly higher temperatures than untreated control rats when subjected to heat. It was

concluded that "AVP could be the etiogenic agent, or at least one of the agents, responsible for convulsions in fever," (Kasting et al., 1981 p.332). Indeed it should be emphasized that since HO Brattleboro rats 100% deficient in AVP did have heat-induced seizures, the presence of AVP is not necessary for the production of such seizures.

The role of AVP in non-febrile seizures:

Although AVP may not be necessary for the production of febrile seizures, this does not deny that it may contribute to them. It is even plausible that AVP is itself sufficient to produce non-febrile seizures. Since the initial studies showing what appeared to be convulsant effects of repeated i.c.v. injections of AVP (Kasting et al., 1980), other studies have attempted to replicate these findings. In the same year, Abood and coworkers were successful in eliciting "severe myoclonic seizures" (Abood, Knapp & Mitchell, 1980, p.193) in rats with i.c.v. injections of 0.1 nmole AVP. Similar results were obtained using lysine AVP.

Burnard and coworkers (1983) also provided support for the notion that AVP has convulsant actions in the brain. In their study, release of endogenous AVP was induced through either hemorrhage of 15% total blood volume or by systemic administration of hypertonic saline. Both techniques have been shown to markedly increase plasma AVP (Schrier et al., 1979). Subsequent i.c.v. injections of AVP elicited seizures

whereas such injections without pretreatment with hemorrhage or saline did not induce seizures. The authors concluded that endogenous AVP was released in the brain during hemorrhage or saline administration, and that such release sensitized the brain in much the same way as repeated i.c.v. administrations of AVP.

Other studies have failed to replicate the convulsant effects of intracerebrally administered AVP. In studies of conditioned avoidance in rats, de Weid (1976) administered AVP intraventricularly but failed to report any seizures. In studies of the autoregulation of AVP neurosecretion, Bhargava, Kulshrestha and Srivastava (1977) injected AVP into the hypothalamus to gauge its affect on AVP release. They also did not report convulsive effects. In a study of intraventricular AVP injections in rats, barrel rotation, as well as a variety of other motor disturbances, was observed (Kruse, et al., 1977). While often misquoted as such, there was no mention of apparent seizures in this study. Lee and Lomax (1983) were unable to produce seizures after i.c.v. administration in the Mongolian gerbil. This is especially remarkable since this gerbil is noted for the ease with which seizures may be elicited. Simple environmental stimuli are often sufficient for the production of seizures in this animal (Bajorek, Lee & Lomax, 1984). In a study attempting to replicate the convulsant effects reported by Kasting and

coworkers, the same doses and volumes of AVP and the same species and injection sites (i.c.v) were used but were ineffective at producing seizures (Corcoran, Cain, Finlay & Gillis, 1984). Repeated injections of AVP in the amygdala were also ineffective (Cain, Plant, Rouleau & Corcoran, 1986).

There are a variety of possible reasons why the convulsant effects of i.c.v. administered AVP as reported by Kasting and coworkers could not be replicated elsewhere. In their original study they cited the failure of other studies, notably that of de Weid (1976), to repeat the injections as the cause for negative results. Kasting and coworkers emphasized that their findings were seen only on the second injection and were consequently the effect of "sensitization" with repeated administration (Kasting et al., 1980, p.318). They also justified the failure of other studies by the size of the animals used. Injections directly into specific brain areas in large-brained animals (such as Bhargava et al., 1977) might not influence regions accessible intraventricularly in rodents. This explanation is questionable on three counts: First, Abood and coworkers (1980) reported convulsant effects but apparently did not find the need to repeat administration in order to get the reported effect. Nowhere in that study was it mentioned that administration was repeated. Secondly, repeated

administration was attempted by Corcoran and coworkers (1984), as well as by Lee and Lomax (1983) without consistent results. Third, the latter groups also used equivalent or smaller animals as those used by Kasting et al.(1980).

Corcoran and coworkers (1984) suggested a number of explanations for the perception of seizures in Kasting's (et al., 1980) and Abood's (et al., 1980) results. They might have mistaken motor disturbances, the presence of which are not disputed, for seizures. Although Corcoran and coworkers as well as Burnard and colleagues (1983) did present electrographic evidence of spiking after i.c.v. AVP administration, such results were unreliable and did not show a progressive increase characteristic of "sensitization." Finally, Corcoran (et al., 1984) also noted electrographic abnormalities in rats receiving only saline, with one animal having seizures, suggesting that Kasting and coworkers might have been observing seizures confounded by the effects of the saline vehicle.

It appears doubtful that AVP acts as a convulsant leading to the progressive development of seizures, yet it remains possible that AVP may modify ictal phenomena indirectly, through the modification of other epileptogenic mechanisms or inhibitory mechanisms.

In order to test the hypothesis that natural levels of AVP may influence seizures of independent etiology, there are

two experimental requirements: (1) a reliable means of inducing seizures in experimental animals and (2) a means whereby the levels of AVP is naturally but reliably altered.

In attempt to satisfy both of these conditions, the experimental method known as "kindling" has been recently implemented by Gillis and Cain (1983, 1986) to determine if a natural deficiency of AVP can influence the development of seizures in rats. Their studies are covered in detail, after a brief review of the kindling method.

The kindling method

The most reliable means of inducing the development of seizures was originally described by Goddard, McIntyre and Leech (1969) as follows:

> The kindling effect is a relatively permanent alteration in brain function which results from repeated electrical or chemical stimulation and culminates in the appearance of electrographic and behavioral convulsions whenever the original stimulus is reapplied.

Many of the fundamental properties of electrical kindling were described by Goddard and coworkers in their pioneering work and by Racine (1972a, 1972b, 1975).

Nonetheless, the kindling phenomenon had apparently been noted much earlier. Tatum and Seevers (1929) reported progressive and lasting increases in the strength of cocaine-induced epileptiform responses. Using cortical electrodes in the dog, Watanabe (1936) may have been the first to describe the electrical kindling effect. Delgado and his colleagues published several reports describing the effect, without recognizing its permanence (Alonso-De Florida & Delgado, 1955, 1958; Delgado & Anaud, 1953; Delgado, Rivera & Mir, 1971; Delgado & Sevillano, 1961).

The complementary studies of Goddard et al. (1969) and Racine (1972a, 1972b) were the first to describe characteristics of and conditions required for kindling. Goddard and colleagues found that: (1) Rats restimulated three months after the initial kindling showed little or no decrement in response, thereby revealing the permanence of the kindling effect. (2) The phenomenon is not the result of tissue damage. (3) The most effective stimulus frequency for eliciting convulsions in kindled rats was found to be about 60 Hz, but no frequencies seemed optimal regarding the rate of kindling. (4) The optimal interval between stimulations was found to be equal to or greater than 24 hours. Shorter intervals required more total stimulations in order to elicit a generalized convulsion. (5) Varying the intensity of the stimulation had little or no effect on the number of

stimulations required to kindle the amygdala, as long as afterdischarges (ADs) were observed. (6) Kindling at a primary site reduces the number of stimulations required to kindle from a secondary site, a phenomenon called "transfer". (7) A minimal current is necessary to evoke such ADs, designated the "threshold" current. (8) Finally, while Goddard and coworkers noted some differences in the rates of kindling and seizure characteristics among various strains of a single species (rat), these workers also first disclosed the remarkable finding that kindling is "a general property of the mammalian brain" (Goddard et al., 1969, pp.317-318).

Following the lead of Delgado and Sevillano (1961) and Goddard et al. (1969), Racine (1972a,b) confirmed that during kindling: (1) the spike amplitude and frequency are increased; (2) the current afterdischarge threshold (ADT) for eliciting an AD is lowered; (3) the behavioral convulsion is increased in strength; (4) electrical ADs are required to produce the kindling effect; (5) all stimulations, both above and below the ADT current, reduced ADTs; (6) stimulations using current below the ADT did not significantly reduce the number of ADs required to kindle with suprathreshold stimulation; (7) kindling at current levels well above the ADT did not alter the rate of kindling compared to kindling done at the ADT.

As a result of these findings Racine concluded that the presence of AD, rather than the electrical stimulus itself, determined the changes seen during kindling.

Racine was also the first to rate motor seizures on a five-point scale, as has now become customary. These stages are seen sequentially during amygdaloid kindling and have been described by Racine (1972b, p282) as follows: stage 1: mouth and facial movements; stage 2: head nodding; stage 3 forelimb clonus; stage 4: rearing; and stage 5: rearing and falling.

The neural mechanisms of kindling:

The mechanism by which kindling has its lasting effects is still hypothetical. Attempts to uncover the mechanism have included studies of electrical, chemical and structural changes in the brain.

Single cell electrophysiology has inspired several lines of inquiry. The first involves a phenomenon denoted "enhancement", which is a change observed in the electrophysiological response of target neurons that receive input from those stimulated directly. The second and more recently described mechanism of seizure development has been termed the "burst response". The evolution of the burst response is seen to involve a mechanism that is separate from enhancement. The burst response is thought to represent a change in the postsynaptic membrane itself. This change is

thought to alter permeability to calcium ions and thereby affect electrical properties of the postsynaptic membrane. In either case, the amplitude of response in the postsynaptic neuron is changed as a result of activity in the presynaptic neuron (Racine, Kairess & Smith, 1982).

Studies of the biochemistry of kindling have found a potential role for norepinephrine and dopamine (Corcoran, 1981), serotonin, gamma aminobutyric acid, glutamate, and certain neuropeptides (Bajorek, Lee & Lomax, 1984) and others.

Attempts to detect structural changes resulting from kindling have not yielded any convincing evidence (Goddard & Douglas, 1975; Racine, Tuff & Zaide, 1975; Racine & Zaide, 1978).

Recent studies have suggested several possible neural mechanisms of kindling involving alterations of neuronal calcium or protein metabolism. Protein synthesis inhibitors appear to have a prophylactic effect against kindling (Morrell, Tsuru, Hoeppner, Morgan, Harrison, 1976; Ogata, 1977; Jonec & Wasterlain, 1979; Cain, Corcoran & Staines, 1987) Nonetheless, the protein synthesis inhibitors used in these studies have a variety of side effects which could be responsible for their observed effects on kindling. Furthermore, their effect could be secondary to the loss of enzymes and the products of enzymatic pathways. Until the

specific proteins involved are identified, their role in kindling remains in question.

An attempt has recently been made to identify specific proteins underlying the electrophysiological changes of kindling. For instance, using immunohistochemical methods, recent studies have suggested that the protein produced by the oncogene c-*fos* shows a transient increase in granule cells of the rat dentate gyrus (Dragunow & Robertson, 1987). The authors suggested the induction of the c-*fos* gene might involve intracellular alterations in calcium, which is known to be redistributed in hippocampal neurons during seizures (Miller, Baimbridge & Mody,1986).

The role of altered calcium metabolism in hippocampal kindling has been related to a long-term depletion in calcium-binding protein (CaBP), as demonstrated using radioimmunoassay methods (Baimbridge & Miller, 1984). These authors suggested that CaBP serves to sequester calcium and that depletion of CaBP is responsible for an increase or redistribution of intracellular calcium, leading to a prolonged hyperexcitable state. On the other hand, Corcoran (1987) has suggested that since depletion of CaBP occurs even when kindling of seizures is blocked by diazepam (Baimbridge & Miller, 1984), and since diazepam has been demonstated to suppress the changes underlying kindling (Racine, Livingston & Joaquin, 1975) CaBP depletion is not directly related to

mechanisms of kindling. One must conclude that the role of CaBP may be clarified only by more sophistocated immunological or chemical techniques in an attempt to alter CaBP and then examine the effects on kindling.

Protein phosphorylation may also underlie changes responsible for kindling. Studies have shown increased phosphorylation (Patel, Marangos, Contel, Gardner, & Post, 1984), decreased phosphorylation (Wasterlain & Farber, 1984) or no changes (Bank, Gurd,& Chute, 1986) in low-molecular weight proteins during kindling. Increased phosphorylation of high molecular weight phosphoglycoprotein(s) was also described by Bank et al., (1986). These studies varied in regards to important kindling parameters and biochemical methodology. Thus, interpreting the role of protein phosphorylation in kindling from these studies is difficult.

Recent studies have attempted to identify specific proteins which undergo phosphorylation during kindling. For instance, lasting phosphorylation of a specific protein, denoted F1, has been shown to be correlated with long term potentiation (LTP) in the dentate gyrus (Routtenberg, 1986). The author has suggested that LTP results from phosphorylation of protein F1, located in synaptic terminals, and that this results from increased activity of protein kinase C.

It is clear that the phosphorylation of specific proteins may well play a role in the neural mechanism of kindling. Recent studies in this area are encouraging.

Kindling in Brattleboro Rats

In kindling studies of Brattleboro rats, Gillis and Cain directed electrodes at the amygdala and pyriform cortex (1983) or the lateral septum and ventral hippocampus (1986). These zones were kindled independently in three types of rats: (a) HO Brattleboro rats, which are unable to synthesize AVP--hence they have diabetes insipidus; (b) heterozygous (HE) Brattleboro rats, which have approximately a 50% plasma deficiency of AVP, and (c) normal control (N) rats consisting of the Long-Evans parent strain.

HO rats did not differ from controls when kindled in the AVP-poor pyriform cortex (Gillis & Cain, 1983). When kindled in the amygdala, HE rats were also close to normal in the development of fully generalized convulsions. However, HO rats required approximately three times as many ADs as controls to achieve a maximal convulsion with amygdaloid stimulation. The amygdala normally contains relatively high concentrations of AVP, presumably due to hypothalamic projections. Prolongation of amygdaloid kindling in AVP-deficient rats was taken to suggest that AVP may be involved with the rate of kindling, especially when kindling is done in areas rich in AVP. However, in kindling in either lateral

septum or ventral hippocampus, both of which are AVP-rich, HO rats kindled most rapidly, the reverse situation from that seen with amygdaloid kindling (Gillis & Cain, 1986). Hippocampus-kindled HE rats were affected similarly, but to a lesser extent. Since some AVP-containing areas of the brain kindled more slowly than controls (amygdala) but others kindled more rapidly (septum and hippocampus), it is not possible to say that the effect of AVP is similar in all areas. Since the pyriform cortex is the only area kindled that normally lacks AVP, it is of interest to see if other areas normally low in AVP kindle at normal rates in Brattleboro rats. One such area of particular concern is the anterior neocortex.

Kindling in the anterior neocortex:

Various studies have provided evidence that kindling of normal animals in the amygdala and pyriform cortex is unlike that in the anterior neocortex. The differences seen in the development of seizures kindled from the anterior (frontal) neocortex were described by Burnham (1978). More electrical current was necessary to evoke ADs in the cortex than in the amygdala. The initial AD was shorter, and more localized, than that observed in limbic kindling. Unlike limbic kindling, the initial AD was accompanied by a behavioral convulsion. Although limbic kindling did eventually elicit behavioral convulsions, these initial convulsions did not

have a purely motor form as seen in the initial cortically kindled seizures. In cortical kindling, significantly more ADs were necessary to produce fully generalized seizures. Finally, the duration of the ADs grew more slowly.

The convulsions elicited by cortical kindling manifested a qualitative change not seen in limbic kindling. The initial seizures were generally clonic in form, followed by a tonic phase, and eventually generalization through a second clonic phase, to create a "tripartite" form (Burnham, 1978). It appears that generalization through the third phase occurred through the eventual involvement of limbic areas such as the amygdala or the pyriform cortex (Seidel & Corcoran, 1986). Since kindling of certain limbic structures is delayed in the HO rat, it remains uncertain whether seizure generalization in the Brattleboro rat would be complete with kindling of the anterior neocortex, since generalization apparently requires the involvement of limbic areas.

It is interesting that in studies of AVP concentrations in the rat brain, Hawthorn and coworkers (1980) distinguished between what they call the frontal lobe (5.6 pg AVP/mg protein) and the anterior cerebral cortex (<2.0 pg AVP/mg protein). This anatomical distinction is unclear. Nonetheless, if we are to take the anterior cerebral cortex as equivalent with Burnham's (1978) "anterior neocortex", its

marked absence of AVP is significant. If generalization of anterior neocortical evoked seizures to limbic regions involves amygdaloid vasopressinergic circuits, it might be observed that only the late clonic phase is abnormal in HO Brattleboro rats. On the other hand, if generalization were dependant on pyriform circuits only, kindling in HO Brattleboro rats might proceed normally. The purpose of the present investigation was to determine whether kindling with electrical stimulation of anterior neocortex is the same in Brattleboro and control rats.

Methods

Subjects

The present study used male adult HE and HO Brattleboro strain rats and normal control male Long-Evans strain rats as controls. Each of these three groups began with 20 adult male rats weighing 200-500 gm. Rats were maintained on a 12/12-h light/dark cycle and provided food and water ad libitum.

Surgical Procedure

All 60 rats were anesthetized with sodium pentobarbital at a dose of 60mg/Kg injected intraperitoneally. The rats were fixed in a stereotaxic instrument and received implantation of bipolar macroelectrodes in the anterior neocortex bilaterally. Electrodes were made of twisted nichrome wire 127um in diameter and insulated with enamel except at their cut tips. The head was positioned as described by Pellegrino, Pellegrino and Cushman (1979), with the incisor bar 5 mm above the interaural line. Electrodes tips were positioned +3.6 mm anterior and 1.5 mm lateral to the skull suture intersect Bregma and 1.7 mm ventral from the surface of the dura mater. Electrodes were connected to amphenol plug strips and cemented in place with a skull cap of dental acrylic anchored with stainless steel screws.

After a recovery period of ten days, kindling was begun in a Faraday cage.

Kindling Procedure

Stimulation was conducted every other day between 11:00 and 14:00h. It was necessary to be consistent in the time of day for kindling since natural levels of AVP are normally 5-8 times greater during mid-day (Reppert, et al., 1981; Reppert, et al., 1987). Electrical stimulation consisted of trains of balanced biphasic square waves at a frequency of 60Hz and with a pulse duration of 1.0 msec. Electroencephalograms (EEGs) were recorded from both electrodes immediately prior and subsequent to all stimulations administered.

During determination of ADTs for each rat, no more than five one-sec stimulations were used per session, grouped no closer than one min apart. Beginning with a level of 100 μA, current was increased by increments of 100 μA until an AD was evoked at either electrode. This was considered the ADT and was used throughout the study for that rat unless the rat became refractory. Refractory rats were those that failed to have an AD when an AD had been observed during a previous session. At the first refractory response, up to four more stimulations were given in increasing increments of 100μA until an AD was evoked. The most recent successful current used was considered the new ADT. If no AD had been obtained by the fifth stimulation in that session, the animal was

considered refractory during that session.

Kindling sessions were continued until one of the following (A,B or C) criteria was reached:

(A) A total of five generalized convulsions (GCs) occurred that conformed to the generalization criterion established prior to kindling. This criterion requires that two out of three of the following characteristics are present during AD in order for the seizure to be considered "maximal:" (1) A stage 4 (rearing) or 5 (rearing and falling) generalized seizure occurred (see Racine, 1972b, p 282, for discussion of this rating scale), (2) the AD lasted for 30 seconds or longer as determined by EEG, or (3) reactivity was seen after the seizure.

(B) 40 stimulation sessions occurred without generalization.

(C) in 3 consecutive sessions there was no AD at currents from 2500 to 3500 μA after kindling had been attempted in both the left, followed by the right, hemisphere electrode.

Upon completion of kindling, all rats were perfused with 10% buffered formalin under an overdose of pentobarbital anesthesia. Brains were frozen and sectioned at 40 to 80 um through the regions of the electrodes and stained with cresyl violet for verification of electrode placements.

For the purposes of the present study, I report data

obtained only from stimulation of the left hemisphere, because the effects of right hemisphere stimulation could have been contaminated by the previous stimulation of the left hemisphere.

Statistics

For comparisons among the three groups I used a one-way analysis of variance. I used the Tukey test (Ferguson, 1971; Godfrey, 1985) with an alpha level of 0.05 for post-hoc comparisons.

Results

Fate of rats

Even though the initial group size was equivalent (n = 20), the final number of rats in the experimental groups differed. This was due to differences in the number of rats that were lost prior to reaching any criterion for completion of the study. The reasons for such losses are listed below with the number of rats lost in each group:

(1) died during surgery: HO = 0; HE = 1; N = 1

(2) died during recovery: HO = 0; HE = 2; N = 1

(3) died during kindling: HO = 2; HE = 0; N = 0

(4) too vicious to use: HO = 1; HE = 0; N = 0

(5) electrode cap unusable: HO = 1; HE = 0; N = 0

(6) pulled electrode cap: HO = 0; HE = 1; N = 6

TOTAL RATS LOST: HO = 4; HE = 4; N = 8

Of those rats that completed kindling (HO = 16; HE = 16; N = 12), only a portion developed generalized seizures. The fate of rats that did not develop generalized seizures was as follows:

(1) no ADs at any currents up to 3500μA in either hemisphere: HO = 1; HE = 2; N = 0

(2) ADs observed but no generalization in 40 sessions: HO = 3; HE = 9; N = 3

TOTAL NUMBER NOT GENERALIZING: HO = 4; HE =11; N = 3

Thus, the total number of rats that developed

generalized seizures were much fewer than originally used:

NUMBER OF RATS THAT DEVELOPED GENERALIZED SEIZURES:

HO = 12; HE = 6; N = 9

Furthermore, it should be noted that those animals which developed generalized seizures only after being stimulated in both hemispheres were excluded from the analysis of kindling rate.

NUMBER OF RATS THAT DEVELOPED GENERALIZED SEIZURES ELICITED BY STIMULATION LIMITED TO ONE HEMISPHERE:

HO = 5; HE = 6; N = 6

Electrode placement

Figure 1 depicts electrode location according to the cortical topography developed by Krieg (1946). Figure 2 provides transverse sections showing the position of electrode tips. In both figures, electrode positions for each rat are coded by symbol shape for experimental group as follows (1) normal controls = diamonds; (2) HE = circles; and (3) HO = squares. The color of the symbols is used to denote the fate of each animal: (1) those rats that developed generalized seizures (criterion A, Methods, p.35) are represented by blue symbols; (2) those that did have AD but did not have five or more generalized convulsions (criterion B) are denoted by yellow symbols; (3) those that never

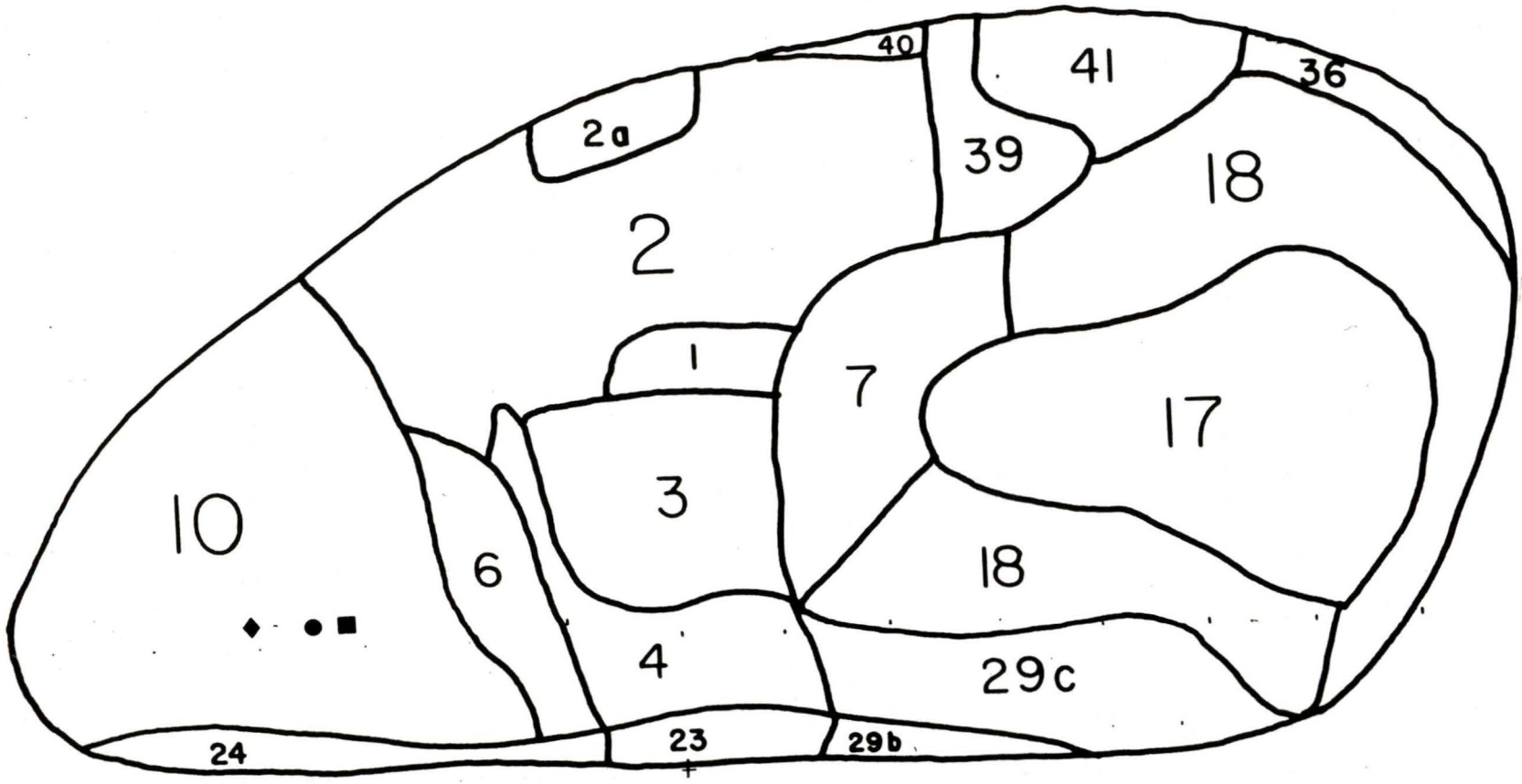


Figure 1. Electrode placement according to the cortical topography of Krieg (1946).

Mean anterior-posterior placements as verified histologically for homozygous (■), heterozygous (●) and Long-Evans normal control rats (◆). Homozygous rats had placements averaging .9mm posterior to those of Long-Evans controls and .3mm posterior to those of heterozygous rats.

Figure 2.

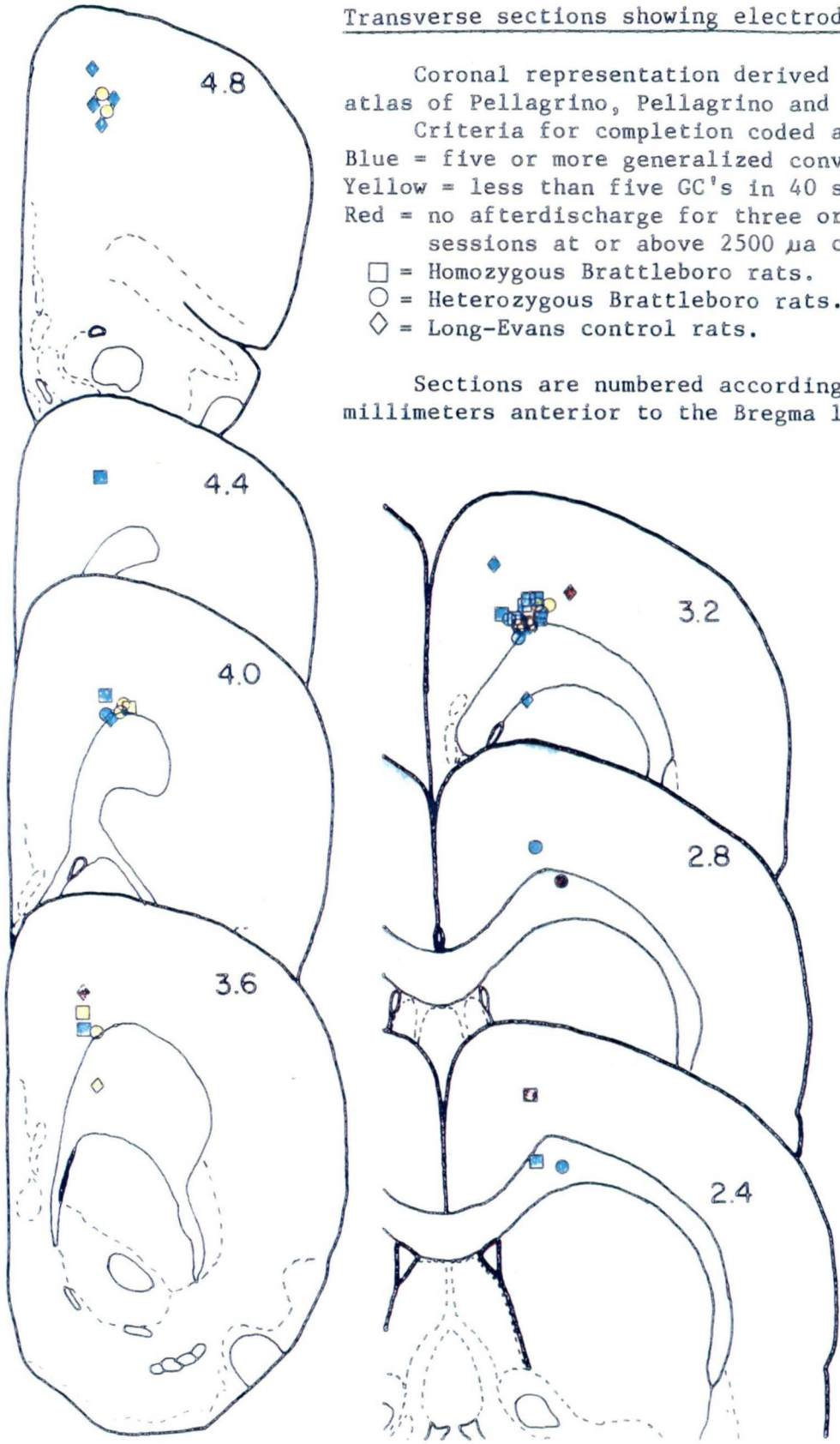
Transverse sections showing electrode placements.

Coronal representation derived from the brain atlas of Pellagrino, Pellagrino and Cushman (1979).

Criteria for completion coded as follows:

Blue = five or more generalized convulsions.
Yellow = less than five GC's in 40 sessions.
Red = no afterdischarge for three or more sessions at or above 2500 μa current.
□ = Homozygous Brattleboro rats.
○ = Heterozygous Brattleboro rats.
◇ = Long-Evans control rats.

Sections are numbered according to the millimeters anterior to the Bregma landmark.



developed generalized seizures before forty sessions or three stimulation sessions of 2.5-3.5 mA current are denoted by red symbols (criterion C). Those placements effective in producing ADs on the first stimulation are denoted by arrows.

As can be seen in Figure 1, the classical subdivision of the rat neocortex suggests that placements fell into the posteromedial quadrant of area 10. Krieg designated Area 10 the "frontal cortical area."

In all groups, electrode placement varied the most along the anterior-posterior axis, less along the dorso- ventral axis and very little along the mediolateral axis. Most tips fell within 1 mm of the cingulum above and/or anterior to the septal nucleus. However, a small number of electrodes ended in white matter of the cingulum or corpus callosum.

There were group differences in the mean position of electrode tips. In comparing the placement of electrode positions with the atlas of Pellegrino, Pellegrino and Cushman (1979), the mean A-P position of electrode tips corresponded to a stereotaxic coordinate of +3.2 mm for HO rats, +3.5 mm for HE rats and +4.1 mm for control rats. That is to say that HO rats had electrodes placed a mean of 0.3 mm posterior to those of HE rats and 0.9 mm posterior to those of control rats. It is conceivable that certain differences in electrode position might be attributed to reduced size in Brattleboro rats (Sokol and Valtin 1982). However, it is

unlikely that size differences led to systematic differences in electrode placement in the present study. Such reductions would have led to electrode positions more anterior for HO rats and more posterior for control rats, just the reverse of that observed.[4] Thus it is likely that observed differences in placement were a result of normal variation.

ADT:

Table 5 lists the mean ADTs for all three groups at the first AD. Initial ADTs averaged 1050 μA for control rats, 767 for HE Brattleboro rats, and 981 for HO Brattleboro rats. These differences were not significant in a one way analysis of variance ($F(2,44)=0.5182$, $p=0.5992$).

Number of Refractory Sessions:

Refractory sessions were those in which the rat failed to have an AD after an AD had been evoked at an equal or lesser current in previous sessions. Five stimulations would be attempted in a session before the rat was considered refractory. If such stimulations reached 2500 μA, the opposite hemisphere was also stimulated up to five times per session.

Table 6 provides the percentage of refractory sessions for each group prior to any stimulations of the contralateral hemisphere. HO Brattleboro rats averaged 47.5%, HE Brattleboro rats averaged 26.1%, and control rats averaged 30.6% refractory sessions. These were not

Table 5.

ADTs at initial AD (μA).

	Brattleboro Rats		Normal
	HO	HE	Control Rats
n	17	15	16
Mean	981	767	1050
Range	100-2100	100-2400	200-2500
Standard deviation	792	727	882
Standard error	198	188	221

Analysis of Variance for ADT at first AD.

	D.F.	Sum of Squares	Mean Squares	F ratio	F Prob
Between groups	2	0.6708	0.3354	0.5182	0.5992
Within groups	44	28.4777	0.6472		
Total	46	29.1485			

Table 6.

Percentage of refractory sessions.*

	Brattleboro Rats HO	Brattleboro Rats HE	Normal Control Rats
n	15	15	15
Mean	47.5	26.1	30.6
Range	03-100	0-100	0-100
Standard deviation	31.2	28.0	39.9
Standard error	8.0	7.2	10.3

*Note: These include only those sessions involving stimulation from that hemisphere in which stimulation was initially effective. After AD's had been evoked from both hemispheres the remaining sessions were excluded from this analysis.

Analysis of variance for percentage of refractory sessions.

	D.F.	Sum of Squares	Mean Squares	F ratio	F Prob
Between groups	2	0.3843	0.1922	1.7201	0.1914
Within groups	42	4.6923	0.1117		
Total	44	5.0767			

significantly different in a one-way analysis of variance ($F(2,42)=1.7201$, $p=0.1914$).

AD Duration:

Table 7 shows the mean duration of seizures (ADD) at the initial AD. Normal controls had a mean duration of 9.8 sec whereas HE and HO rats had 12.4 and 16.0 sec respectively. One way analysis of variance demonstrated a significant difference among groups ($F(2,47)=4.4178$; $p=0.0175$), for which a post-hoc comparison (Tukey's method, see Ferguson, 1971, p 274) showed that only the HO and control groups were significantly different at the 0.05 level.

Kindling rate

It is remarkable that in all groups, few Stage 1 or 2 seizures were observed. This contrasts with the stepwise progression through all stages during limbic kindling (Burnham, 1978). Table 8 shows the mean number of ADs seen prior to the first generalized seizure (GC) in all groups. Differences were not significant in a one-way analysis of variance ($F(2,14)=0.2344$; $p=0.7941$). HO and HE rats required means of 6.2 and 5.5 ADs respectively, whereas control rats required 4.7 ADs. In all groups, more than four submaximal seizures were required to reach the fifth GC. Thus, by the fifth GC, rats had required more than 10 ADs on average.

Table 7.

ADDs at initial AD (sec).

	Brattleboro Rats HO	Brattleboro Rats HE	Normal Control Rats
n*	17	15	17
Mean	16.0	12.4	9.8
Range	4-32	4-32	9-32
Standard deviation	6.12	7.39	4.7
Standard error	1.49	1.84	1.14

*Note: These numbers include several animals which later died or lost electrode cap-plugs and therefore did not finish kindling.

Analysis of variance of ADD at first AD

	D.F.	Sum of Squares	Mean Squares	F ratio	F Prob
Between groups	2	333.2712	166.6356	4.4178	0.0175
Within groups	47	1772.8088	37.7193		
Total	49	2106.0800			

Table 8.

Number of ADs to generalization.

	Brattleboro Rats HO	Brattleboro Rats HE	Normal Control Rats
n	5	6	6
Mean	6.2	5.5	4.7
Range	1-33	1-29	11-29
Standard deviation	3.7	4.5	2.7
Standard error	1.7	1.8	1.1

Analysis of variance of number of ADs until generalization

	D.F.	Sum of Squares	Mean Squares	F ratio	F Prob
Between groups	2	6.4843	3.2422	0.2344	0.7941
Within groups	14	193.6333	13.8310		
Total	16	200.1176			

Since the criteria used for defining generalization may alter the mean number of ADs required for generalization, I recalculated the number of ADs prior to generalization according to the criteria of both Seidel and Corcoran (1986) and of Burnham (1978). These results are listed on Tables 9A and 9B and demonstrate that these more stringent criteria for generalization result in the appearance of slower kindling due to the greater number of ADs required to reach such criteria.

Seidel and Corcoran (1986) considered seizures to be generalized only when all of the following conditions had been observed: (a) an AD duration increase of over 40% over three consecutive ADs, (b) reactivity ("postictal hyperirritability"), (c) rearing and falling (Stage 5 seizure). In the present study, such criteria yielded a mean number of ADs prior to generalization of 17.7 for HO rats (n=3), 20.3 for HE rats (n=6) and 19.0 for normal controls (n=3). (See Table 9A.)

In at least one of his studies, Burnham (1978) considered any "rearing and falling convulsion" over 20 seconds to be maximal and calculated the rate of kindling on this basis. In the present study, I found the mean number of ADs required to reach this criterion were 11.4 for HO rats (n=5), 15.7 (n=6) for HE rats and 14.0 (n=4) for normal

Table 9A.

Number of ADs to generalization using the criteria of Seidel and Corcoran, 1986.

	Brattleboro Rats HO	Brattleboro Rats HE	Normal Control Rats
n	3	6	3
Mean	17.7	20.3	19.0
Range	15-19	2-33	11-26
Standard deviation	2.31	10.57	7.94

Table 9B.

Number of ADs to generalization using the criteria of Burnham, 1978.

	Brattleboro Rats HO	Brattleboro Rats HE	Normal Control Rats
n	5	5	4
Mean	11.4	15.2	13.75
Range	3-19	2-24	6-20
Standard deviation	6.99	8.53	6.13

controls. (See Table 9B.)

Discussion

Fate of rats

The loss of rats prior to and during kindling has important implications for the evaluation of the results of the present experiment. It is remarkable that HO and HE Brattleboro rats survived surgery and kindling as well as their normal counterparts. Given the overall poor metabolic condition especially of HO rats, this was not to be expected.

Other noteworthy findings concerning the fate of rats included the fact that whereas HO and control groups each had only one animal that had ADs but failed to generalize, the HE group included nine in this category. This was largely responsible for the fact that the HE group included only 5 rats meeting the criterion of five generalized seizures.

Electrode Placement

Figures 1 and 2 reveal that all placements fell within the posteromedial third of the anterior neocortex (Area 10) with the exception of a few rats that had electrodes in the premotor region (Area 6). The whole of this region is involved intimately with motor behavior and is organized somatotopically on both the anterior-posterior and medial-lateral dimensions. A dense projection is received from the mediodorsal and ventrolateral nuclei of the thalamus via the

thalamic radiations (Brodal, 1981, pp. 94-99). Additional afferents arrive from both inter- and intrahemispheric pathways via the corpus callosum and cingulum respectively. Efferent connections consist of reciprocal cortical pathways and motor output via the extrapyramidal and pyramidal outflow.

The connectivity of regions kindled accounts for several of the seizure characteristics seen in the present study. For instance, it is not surprising that initial electrographic seizures are accompanied by motor seizures. This is to be expected from an area that is the primary source of cortical motor outflow. Also, the relative paucity of direct connections to limbic cortex may account for the slow development of limbic generalized seizures in comparison to kindling of such structures as the pyriform cortex or amygdala.

An unexpected observation was that electrode placement in control rats were more anterior than in Brattleboro rats. Furthermore, electrode placement in all groups varied from other studies of kindling of anterior neocortex. The stereotaxic coordinates used were 0.1 mm anterior to Altman and Corcoran's (1983) and 1.1 mm anterior to those used by Burnham (1978). However, actual placements were different, as judged by the atlas of Pellegrino et al. (1979): The A-P placement for HO rats was 0.3 mm posterior to the coordinate

used by Altman and Corcoran and 0.7 mm anterior of that used by Burnham. For HE rats, the A-P placement was identical to Altman and Corcoran's coordinate but 1.0 mm anterior to that of Burnham. For the control group, placement was 0.6 mm anterior to Altman and Corcoran's coordinate but 1.6 anterior to that of Burnham. Despite these differences in electrode placement between experimental groups and between studies, the areas kindled are not significantly different in terms of functional or connective organization. Consequently, differences in the mean position of electrode tips should not have accounted for any differences observed in kindling.

Electrode tip positions near the cingulum (juxtacingular) were the only positions effective in producing behavioral convulsions on the first stimulation in a study of the efficacy of a wide variety of cortical and limbic electrode placements (Goddard et al., 1969). In the present study, six rats had ADs on the first stimulation, hence at a current of 100 uA. One of these was a HO Brattleboro rat, four were HE Brattleboros, and one was a control rat. Twenty-three rats had ADs during the first session. That is, such ADs occurred in five or fewer stimulations with initial ADTs at or below 500 uA. Whereas such rats seemed slightly more likely than the others to have electrodes placed near white matter of the cingulum or corpus callosum, this did not appear significant on inspection. For

instance, in the HO rat having an AD on the initial stimulation, the distance of the electrode to white matter of the cingulum or corpus callosum was between 1.0 and 1.5 mm. Nonetheless, the fact that 10% of all rats in the present study had ADs on the initial stimulation is consistent with earlier findings that stimulation of juxtacingular cortex readily evokes behavioral convulsions without prolonged kindling. The ease with which behavioral convulsions are evoked in anterior neocortical kindling is presumably due to this area's being an important pathway in the behavioral expression of cortical seizures. Thus it is not surprising that AD corresponded almost exactly with the behavioral seizure, both in AD onset and duration. Only on very few occasions was AD activity seen within the routine minute of observation after the behavioral convulsion ended.

ADTs

Initial ADTs (Table 5) were similar to some--but not all--other studies of cortical regions in rats. In the study of Altman and Corcoran (1983), the mean ADT was 1029 μA in control rats. In the present study, there was an almost identical initial mean ADT for normal controls (1050 μA) and a similar ADT for HO Brattleboro rats (981 μA). Nonetheless, Burnham (1978) reported ADT's of 340 μA for kindling in cortical Area 6 (Krieg, 1946), the premotor cortex, which

lies slightly posterior to most placements in the present study. Altman and Corcoran regarded their high ADTs to be the result of superficial electrode placement in agreement with their communications with Burnham (Altman & Corcoran, 1983, p 175). As a result of these remarks, in the present study the electrodes were placed 0.3-0.5mm deeper than in that of Altman and Corcoran[5]. Rats with deeper electrodes did not seem to require less current in the present study. This is consistent with the early findings of Goddard et al. (1969), who found that a juxtacingular placement in motor cortex using A-P coordinates of 2.6 mm anterior to Bregma, or approximately 0.7 - 1.5 mm posterior to that verified in the present study, required very large currents: 5,000 or 10,000 μA. Despite the need for large currents in this area, Goddard and coworkers provided evidence that juxtacingular stimulation was most reliable in producing an AD on the first stimulation. Finally whereas superficial placements do not seem to require greater currents to evoke an AD, Goddard did provide evidence that juxtacingulum stimulation was most reliable in producing an AD on the first stimulation in addition to evidence that superficial placements take longer to kindle.

As in the present study, Altman and Corcoran (1983) did not attempt to reduce intensity of stimulation during kindling. It is therefore likely that their currents were

also higher than the true ADT. Nonetheless, ADT's of control rats were lower in their study than those of normal control rats in the present study, due to other methodological differences discussed below.

The method used for determining the ADT in the present study differed from previous cortical kindling studies in at least four ways: (1) Current levels were increased by constant increments of 100 μA until an effective stimulus was found. Other studies used either smaller incremental changes (10 μA in the studies of Gillis & Cain, 1983; 1986) or a fixed percentage of the previous current (currents were doubled in the study of Burnham, 1975 and Racine, 1972a). (2) Up to five stimulations were given per session at one session every other day. Gillis and Cain (1983,1986) apparently chose to raise the current indefinitely until an AD of at least 5 s duration was evoked on the first day. Burnham (1975) and Altman and Corcoran (1983) apparently used Racine's (1972a) method of giving only one stimulation per day during ADT determination. (3) Whenever stimulation failed to elicit an AD at or above currents that were previously effective (refractory episode), the current was increased until an AD was evoked or until five stimulations in 100 μA increments had been given in that session. Seidel and Corcoran (1986) have also described an unusually high rate of refractory sessions in frontally kindled rats. (4)

No reductions of current were made in order to "fine tune" the ADT by incremental reductions (as described by Racine 1972a, p 270).

The ADT determination procedure as described for the present study might easily have led to stimulation at levels that were markedly above the current necessary to evoke AD for any given session. The practice of raising current during refractory states was chosen in order to begin kindling as soon as possible in the knowledge that suprathreshold current levels do not alter amygdaloid kindling rates (Racine, 1972a).

The frequency of refractory episodes

With the exception of the study of Seidel and Corcoran (1986), the frequency of refractory kindling episodes is rarely reported. Thus, it is difficult to know if the significant number of refractory sessions seen in their study, as well as in the present study, is typical of cortical kindling (Table 6). Furthermore, other workers have not reported their method of dealing with such refractory sessions. Alternatives might include: (1) Restimulating the animal later the same day. (2) Restimulating at the next scheduled session. (3) Removing the animal from the study, especially if the refractory response continued. (4) Assuming an increase in ADT and thus increasing the

intensity of stimulation.

The relation of ADT to the frequency of refractory episodes

If the refractory state is merely a temporary state that is insensitive to current levels, increasing the intensity of stimulation could result in current levels well above the actual ADT. It seems likely that the refractory state was independent of intensity of stimulation in the present study, because seizures were rarely evoked by raising the current during a refractory session. Furthermore, seizures were often seen again on the first stimulation two days later, even on those occasions when the original current level was accidentally used. Finally, after several rats had developed generalized seizures, an attempt was successfully made to evoke seizures at reduced current levels. Consequently, in rats that had been refractory one or more times, the current levels were probably well above the actual ADT.

Several studies have suggested that kindling may be inhibited by prior repeated stimulations. This finding is peculiar since it suggests that repeated stimulations may not only lead to the progressive development of evoked seizures, but that electrical stimulations may also lead to the inhibition of such seizures. It appears that subtle but crucial differences in stimulation protocols are responsible. Working with amygdaloid kindled Long-Evans rats, Shao and

Valenstein (1982) showed that it is possible to stimulate above the ADT without evoking seizures by "a regimen of small incremental increases in current intensities delivered at 6.5 s interstimulus intervals," (p. 391). Their finding is provocative since this is the approximate regimen used in the present study when determining ADT. Shao and Valenstein reported that the inhibitory effect resulting from this procedure lasted at least 24 hours. Hence, massed stimulations as used to determine the ADT in the present study could account for refractory episodes for a given session 48 h later. Furthermore, it is also interesting that in both the present study and that of Shao and Valenstein, there was a high frequency of "hyperresponsiveness and aggressiveness" after kindling sessions. Potential mechanisms that might link massed stimulation with the refractory and hyperresponsive states remain uncertain, but in any case the present results suggest that massed stimulation should not be used during determination of ADT.

ADD

It is interesting that HO Brattleboro rats had a significantly longer initial AD than control rats but not HE Brattleboro rats. This finding corresponds to AVP levels in the three groups in that significantly longer ADs are seen with only complete deficiency of AVP. This result

contradicts the hypothesis that endogenous AVP enhances the development of seizure activity. Rather, it suggests that where AVP is absent, seizures are prolonged.

The relation of ADD to ADT

It has been suggested that kindling at higher currents leads to shorter ADs since the two are correlated (Le Gal La Salle, 1982, p.40). It is likely that since HO rats were more often refractory in the present study, their ultimate kindling took place at suprathreshold currents well above those of HE and control rats. Nonetheless, HO rats did not show a reduced AD duration as Le Gal La Salle's theory might have predicted. If the theory is correct and if it applies to Brattleboro rats, the notable prolongation of ADs in HO Brattleboro rats could even be underestimated by the present study due to the counteracting effect of high kindling currents.

Rate of kindling

It is worthwhile comparing the rate of kindling of control rats in the present study with other studies of anterior cortical kindling. In the study by Burnham (1978), the mean number of ADs prior to the first "rearing and falling" convulsion of over 20 seconds duration was 37.3. The number of ADs to such a seizure over 30 seconds duration was 23.4 in the study by Altman and Corcoran (1982). Seidel

and Corcoran (1986) found a similar figure for ADs to generalization, 24.2, but again they used unique criteria based on relative, rather than absolute, duration of the ADs. In the present study, the mean number of ADs prior to generalization was 4.7 in control rats.

It is remarkable that so few ADs were required in the present study. This may have been because the criterion for defining generalized seizures was more liberal than each of the comparable studies. Unlike the studies by Altman and Corcoran (1983) and Seidel and Corcoran (1986), the criterion used in the present study considered any seizure longer than 30 seconds duration and accompanied by reactivity to be a generalized convulsion. Furthermore, unlike the previous studies, stage 4 or 5 seizures of any duration accompanied by reactivity were also considered to be generalized. Of those control rats that developed generalized seizures in the present study, five would not have been considered to have done so according to the criteria of either Altman and Corcoran (1983) or of Seidel and Corcoran (1986). Similarly, Burnham's criteria (1978) would have excluded three rats considered to have generalized in the present study. Thus the apparently rapid rate of generalization in the present study is, I suggest, due in part to classifying animals as generalized at an earlier point in kindling than would have been the case using the

criteria of other studies.

Comments on methodology

There has been inconsistency among studies concerning the duration of AD that has been considered "cortico-generalized". For instance, Burnham (1978, p.508) stated "Any cortically elicited seizure which lasted 20 seconds or more and which involved a 'rearing-falling' convulsion has been scored as 'cortical-generalized'". Yet in a later article, the same author defined a cortico-generalized seizure as " . .a cortex-triggered seizure. . .(that) consisted of an electrographic discharge which lasted 30 or more seconds and was accompanied by a convulsion which involved an episode of 'late' (rearing-falling) clonus" (Burnham 1980, p.163). Indeed, whereas the study of Altman and Corcoran (1983) used the latter criteria of a 30sec AD, they compared their results with Burnhams' earlier report using a 20sec criterion. While such a distinction in AD duration may not reflect markedly different seizure states, it can affect the count of the number of ADs prior to generalization when used as a criterion. This is illustrated in this study by the fact that when different criteria were used to calculate the number of ADs to generalization, results are altered even with regard to which group required the most ADs. (Albeit results using alternative criteria were

not analyzed statistically due to the small numbers of subjects and such differences may not have been significant). One must conclude that variability among studies may reflect methodological, rather than biological differences, as noted above.

Differences in methodology are not limited to criterion for AD duration in cortico-generalized seizures, but also to electrode placement, rat strain, and criteria for inclusion or exclusion of rats. For instance, in the present study all rats failing to generalize by their 40th session were removed from further study. If withdrawl had been delayed until the 50th session, however, it is likely some of the rats would have achieved a stage 5 (cortico-generalized, or rearing-falling) seizure. This would have resulted in a higher mean for the number of ADs prior to the first stage 5 seizure. Unfortunately, not only did other studies fail to report how refractory rats were dealt with, but they also did not mention whether a criterion number of sessions was used to eliminate rats from the study if they failed to generalize. In the case of Burnham (1978), the number of rats that failed to generalize was quite small. Burnham (1975) suggested that his methodology was similar to that of Racine's suprathreshold technique (1972a,b), in which rats were subjected to electrical stimulation trials until a "full motor seizure was evoked". Apparently Burnham stimulated at

least some of his rats for up to 200 sessions and found that even with such persistence, a small number of rats failed to develop generalized seizures (Burnham, 1978, p. 514). It therefore appears that stimulation might well have been continued beyond 40 sessions for many rats in his study, with the consequence that when the mean number of sessions to generalization were calculated, these rats were included. This would have led to a higher calculated mean.

Implications for the role of AVP in seizures

In the present thesis I demonstrated that anterior neocortical kindling in HO and HE Brattleboro rats does not differ from cortical kindling in control rats in terms of rate of generalization, ADT, or percentage of refractory sessions. However, initial ADDs in Brattleboro rats were significantly longer than in controls. Collectively these results suggest that AVP is not only not required for anterior neocortical kindling, it may actually antagonize aspects of cortical epileptogenesis. This conclusion is clearly at variance with the hypothesis that AVP plays a facilitory role in seizures in general and kindling in particular.

Three lines of research have linked AVP with epileptogenesis: kindling in Brattleboro rats, the effects of central infusions of AVP, and evidence of AVP's involvement

in febrile convulsions. Gillis and Cain (1983, 1986) have examined the characteristics of kindling with stimulation of areas normally rich in AVP (amygdala, lateral septum, hippocampus) or normally deficient in AVP (pyriform cortex). They found that HO Brattleboro rats displayed either retarded or accelerated rates of kindling when stimulated in AVP-rich areas, whereas the rate of kindling with stimulation of pyriform cortex did not differ from that seen in controls. The present experiment extends their analysis to kindling of anterior neocortex, a second region that in control rats is deficient in AVP, and the results indicate that kindling of such regions proceeds without impediment in the absence of AVP. The finding that kindling of AVP-rich regions is either retarded (Gillis & Cain, 1983) or accelerated (Gillis & Cain, 1986) in Brattleboro rats clearly suggests that there is no simple relation between kindling of seizures and the presence of AVP. If AVP does play a role in kindling its contribution is not obvious from studies using Brattleboro rats.

A second piece of evidence suggesting that AVP might be causally involved in epileptogenesis comes from studies demonstrating that repeated central infusions of AVP can kindle seizures (Kasting et al., 1981; Burnard et al., 1983). However, the reliability of this finding is called into question by the failure of investigators in other laboratories to replicate the effect (Cain et al., 1986;

Corcoran et al., 1984). Thus kindling with infusions of AVP does not offer firm support for the idea that epileptogenesis depends on AVP.

The final evidence suggesting that AVP might be involved in epileptogenesis has come from investigations of febrile convulsions in Brattleboro rats and in control rats treated with anti-AVP antiserum (Kasting et al., 1981). Absence of AVP or treatment with antiserum led to a significant increase in the threshold temperature for eliciting febrile convulsions, clearly pointing to a role for AVP. The fact that febrile convulsions nonetheless occurred in the absence of AVP suggests, however, that the role of AVP is only modulatory and that it is not essential for epileptogenesis.

Thus it appears that AVP is one of a number of substances that can modulate susceptibility to seizures. It is not, however, essential for any form of epileptogenesis, particularly kindling. The present results confirm that kindling can proceed at control rates in the absence of central AVP. These results offer little encouragement for further investigation of the role of AVP in the development of seizures.

Footnotes

1. Antidiuretic hormone (ADH) and vasopressin (VP) are now considered to be equivalent under most contexts. The former term has been in use for decades and denotes the function, rather than the molecular identity of the substance. This distinction results from the historical method of assaying the presence of ADH through its biological properties in standardized preparations. Other natural peptide fragments besides VP are known to possess antidiuretic activity, and may occasionally account for the presence of ADH activity in biological assays. When the molecular structure of antidiuretic hormones were first determined in the 1950's, the more specific nomenclature was instituted, becoming preferred by the mid-1960's. (See Schroder and Lubke, 1966, pp 281-374.)

2. Calculation of these dosages is based on the standard equivalent of 1 mg = 2.1 "Units" active AVP accurate -15% to +20% (U.S. Pharmacopeia XX, The National Formulary XV, 1979).

3. For the purposes of this paper, the terms "convulsion" and "seizure" are used interchangeably without any loss in accuracy. It should be emphasized that "convulsion" is a term used to describe behavioral manifestations that are well known. "Seizure" is often used in the same context. However, "seizure" is a broader class of phenomena including any widespread paroxismal discharge of cortical neurons. If such seizures occur outside of areas involved with motor function, they occur in the absence of convulsions. Convulsions may not occur without an electrographic seizure. Stated simply, convulsions are a behavioral manifestation of electrographic seizures involving motor pathways.

4. Since the A-P coordinate is measured from the Bregma landmark (an intersection of two principal skull sutures, a point having uniform position in most individuals), which lies posterior to the anterior neocortex, a given coordinate anterior of this landmark --as used for anterior neocortical electrodes--would traverse a larger portion of brain for smaller brained animals, such as might be the case for HO rats. The reverse was observed in that electrode positions were most posteriorly placed in homozygous rats.

5. Comparison of the D-V coordinates used in the present study with that of Altman and Corcoran is complicated by the fact that they measured from the surface of the skull and I measured from the dura. If the skull and extradural space is considered to be between 0.8 and 1.0 mm thick, coordinates used in the present study are 0.3-.5 mm deeper than in their study.

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