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The Effects of Basal Forebrain N-methyl-D-aspartic Acid Lesions on Self-Stimulation of the Lateral Hypothalamus and Ventral Tegmental Area

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A Thesis

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ABSTRACT

The Effects of Basal Forebrain
N-methyl-D-aspartic Acid Lesions on Self-Stimulation
of the Lateral Hypothalamus and Ventral Tegmental Area

Andreas Arvanitogiannis

A major goal for research on intracranial self-stimulation is the identification of the directly-activated cells subserving the powerfully rewarding effect produced by electrical stimulation of the medial forebrain bundle (MFB). Electrolytic lesions of the anterior MFB have been previously shown to attenuate the rewarding impact of stimulating more caudal MFB sites. In the present study, excitotoxic lesions were employed to determine the relative contribution of somata or fibres of passage contributing to that effect. Changes in reward efficacy were inferred, at three currents, from lateral displacements of the function relating the rate of responding to the number of stimulation pulses per train. Collection of baseline data from stimulation sites in the lateral hypothalamus (LH) and the ventral tegmental area was followed by the injection of 70nmol of N-methyl-D-aspartic acid via cannulae aimed at basal forebrain sites. Three subjects were injected with vehicle and served as controls. In 5 out of 15 subjects, lesions encompassing the lateral

preoptic area, anterior LH, and substantia innominata resulted in long lasting, large increases (0.2-0.4 $\log_{10}$ units) in the frequency threshold for self-stimulation. Seven other rats with similar or more dorsally located damage showed substantial, transient increases in threshold. Vehicle injections did not affect behaviour. Varying degrees of demyelination were seen, mostly removed from the electrode tip, and in locations that varied substantially across subjects. Although open to more than one interpretation, results support the notion that somata in the basal forebrain give rise to some of the directly-activated fibres subserving self-stimulation of the MFB.

Acknowledgements

This is an opportunity for me to thank my thesis supervisor Dr. Peter Shizgal who believed in me at a difficult time in my career path and has provided me with continuous support and encouragement. He not only taught me about intracranial self-stimulation or, more generally, neuroscience, but provided me with a model of what a scientist should try to be. I hope I can imitate his high standards and style in my scientific undertakings. It is clear to me that this thesis has improved considerably as a result of his thoughtful suggestions.

Dr. Meg Waraczynski has been a great teacher. She patiently supervised my work at the initial stages of this project and provided me with the tools to become autonomous. I wish her all the best in her career.

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At this point, the thought of my loving wife Myriam overwhelms me with emotion. What can I say to express how much she has offered me? How can I repay her patience, understanding, endless hours of help, insights, love...

I gratefully acknowledge the financial support I received throughout my Master's from the "Alexander S. Onassis" Public Benefit Foundation.

Dedication

I would like to dedicate this thesis to my father Spiros, my mother Yianna, and my brother Alexis. Without their love and support, I would never have come so far...

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In order for an animal to survive, its behaviour must be constantly responsive to external and internal requirements. A multitude of responses and actions are initiated to address these requirements. Motivational states reliably guide the animal towards goals that contribute to homeostasis and ecological fitness. How motivational systems influence behaviour and how goals are evaluated in the central nervous system have always been fundamental questions in the study of brain and behaviour.

The discovery of self-stimulation by Olds and Milner (1954) was a major milestone in the development of a physiological approach to the study of motivation. They showed that rats can be trained to perform responses, such as lever-pressing, to obtain electrical stimulation of deep brain structures. The behavioural effect of such stimulation is so powerful that animals working to receive it may forego the procurement of necessities such as food, even in states of severe deprivation (Frank & Stutz, 1984). Thus, it has been suggested that the neural system responsible for intracranial self-stimulation plays a role in both adaptive behaviours such as feeding (Hoebel & Teitelbaum, 1962; Hoebel, 1969; Rolls, 1972) and in maladaptive behaviours such as self-administration of drugs of abuse (Wise, 1980). Furthermore, Gratton and Wise's (1988a, 1988b) results suggest that a common set of directly-activated neurones contributes to BSR and stimulation-induced feeding.

Recently, Conover and Shizgal (1992) showed that brain stimulation and gustatory rewards are evaluated in a common system of units and combined in an agonistic fashion.

Together, these observations suggest a close relationship between the neural systems subserving brain stimulation reward (BSR) and those important for day to day motivated behaviours. A more direct approach to assessing such a liaison would be to use unit recording in conscious animals, making it possible to correlate the activity of cells activated by rewarding stimulation with behaviour directed towards natural rewards. Application of this technique requires that at least some of the directly activated neurones responsible for BSR be identified. The experiments described below are aimed at furnishing information crucial to the identification of such cells. Localization of these somata would permit recording experiments to be carried out as well as experiments aimed at tracing the inputs and outputs of these somata.

The elucidation of the neuroanatomical organization subserving BSR will not only lead us to a more complete understanding of motivation towards goals critical for survival, it will also afford neuroscientists with a cellular model for studying the neural basis of learning in vertebrates (Gallistel 1991). This idea stems from evidence that self-stimulating animals store an enduring record of the

magnitude of the rewarding signal generated by the stimulation they previously received (Gallistel, Stellar, & Bubis, 1974). Future behaviour is presumably guided by this memory trace.

The initial strategy employed to investigate the system subserving BSR was to map the brain sites that support self-stimulation. Stimulation of numerous regions evoked BSR, the medial forebrain bundle (MFB) yielding a very effective and insatiable self-stimulation behaviour. Accordingly, many BSR investigators have concentrated their efforts on determining which neurones within the MFB subserve the rewarding effect.

The MFB is a heterogeneous structure comprising at least 50 discrete ascending and descending fibre systems (Nieuwenhuys, Geeraedts, & Veening, 1982). Amid the complexity, several techniques have been applied to narrow down the list of candidate neurones. Using 2-Deoxy-D-glucose along with autoradiography (Gallistel, Gomita, Yadin, & Campbell, 1985) or immunohistochemical labelling of the Fos protein (Shizgal, Arvanitogiannis, Conover, & Pfaus, 1993), neurones activated by rewarding stimulation were visualised. Structures containing activated neurones are mainly located in the basal forebrain. Although appealing and useful, these methods are insufficient for identifying reward-related neurones because they fail to distinguish between these cells and other stimulated neurones. The psychophysical approach

is aimed at differentiating reward-related neurones from the pool of activated neurones. Behavioural estimation of the axonal trajectory, the size, the excitability properties, or the degree of myelination introduces some constraints as to the possible identity and location of directly-activated reward neurones ("first-order" or "first-stage" neurones).

Psychophysical studies: portrait of the reward substrate

To gain a foothold on the architecture of the circuitry underlying a particular behaviour, it is useful to establish the quantitative characteristics of the processes that underlie it. This can be accomplished by changing one parameter of the stimulus that controls the behaviour and studying the degree to which another parameter must be adjusted in order for the behaviour to remain constant. In the case of BSR, electrical stimulation parameters are commonly traded-off against one another in order to maintain a criterion level of lever-pressing. For example, the strength-duration function is determined by trading-off the current required to produce a given level of behaviour versus the duration of the stimulating pulse. The resulting strength-duration function provides clues as to the type of fibres that are included in the first-stage.

It is important to bear in mind, that the property of monotonicity is what renders psychometric measurements valid

indices of the characteristics of the first order neurones. If the function relating behavioural output to stimulus input is monotonic over some unique range, then behaviourally based trade-off functions are equally descriptive of all the stages of the substrate preceding, and including the stage that combines the effects of varying the input parameters (Gallistel, Shizgal, & Yeomans, 1981). Thus, data obtained from analysis at the behavioural level are pertinent to analysis at the neuronal and network levels. In the case of BSR, a broken line function best describes the interactions between electrical stimulation parameters and output vigour. The rat initiates lever-pressing after some critical value of stimulation is reached with further input increases resulting in a proportionally higher rate of lever-pressing, up to a performance ceiling. In other words, the input-output function grows monotonically over a unique range of stimulation strength.

Trade-off experiments provide the backbone knowledge of the electrophysiological and anatomical characteristics of the first-order neurones. The refractory periods of the reward-relevant neurones and their conduction velocities are inferred from the results of double (C-T) pulse experiments, in which the number of pulse pairs required for criterial behaviour is traded-off with the intrapair intervals (Yeomans, 1979; Bielajew, & Shizgal, 1982). To infer refractory periods, both the C and the T pulses are delivered

through the same electrode. Conduction velocity is inferred from the behavioural adaptation of the collision technique which consists of delivering the two pulses through separate electrodes implanted at sites supporting self-stimulation. Within the MFB, such sites include the ventral tegmental area (VTA) and the lateral hypothalamus (LH). Using the collision test, Shizgal, Bielajew, Corbett, Skelton, & Yeomans (1980) have found that first-order neurones link the LH and the VTA. Together, these important experiments suggest that the substrate for BSR in the MFB consists, at least in large part, of fine, myelinated fibres coursing through the LH and the VTA. In at least some of the directly activated fibres subserving MFB reward, the behaviourally relevant direction of conduction is rostro-caudal, suggesting that basal forebrain structures are possible sources of reward-relevant axons (Bielajew & Shizgal, 1986). The idea that first-order neurones originate in the basal forebrain and give rise to axons that descend through the MFB towards the tegmentum has come to be called the "descending path" hypothesis of MFB self-stimulation. It is worth mentioning that neurones with the characteristics inferred from the psychophysical data are, indeed, activated by rewarding MFB stimulation (Rompré & Shizgal, 1986; Shizgal, Schindler, & Rompré, 1989).

The lesioning approach

Psychophysical data sketch a portrait of the first-stage

neurones. In turn, electrophysiological recording identifies "candidate cells", that is, cells that are activated by stimulation of the MFB and that exhibit properties expected for first-order neurones. Unfortunately, the profile of candidate neurones is not necessarily unique to those that carry the reward signal away from the tip of the electrode. Lesion experiments are invaluable in establishing which of the candidate neurones are in fact a part of the reward substrate. A decrease in the magnitude of the rewarding effect following damage to a nucleus containing candidate cells provides the evidence needed to establish this site as a "reward nucleus".

The following example clearly illustrates the important role that lesions play. The promising electrophysiological portrait of cells recorded from the septal complex (Rompré & Shizgal, 1986; Shizgal et al., 1989) along with evidence of increased metabolic activity throughout the septal complex following rewarding stimulation of the MFB (Gallistel et al., 1985) pointed to a possible role for this region in self-stimulation. Nevertheless, knife-cuts disconnecting the septal areas from the MFB failed to produce changes in rewarding effectiveness (Waraczynski, 1988). Thus, cells in the septum only resemble reward-relevant neurones; evidence from lesion studies prompts us to continue our search elsewhere. In that respect, successful lesions serve to establish the importance of structures already proven to

contain candidate neurones.

Curve-shift scaling

A serious conceptual limitation of past lesion experiments involves the use of crude behavioural measures. Unfortunately, the most common way of evaluating the effects of lesions on MFB stimulation reward was to compare self-stimulation rates before and after the lesion. If after damaging a brain structure a decrease in the operant response of the rat is observed, it is tempting, but nonetheless risky, to ascribe a putative role for the structure in BSR. Damage to the brain can alter the rate of operant responding for stimulation of fixed parameters due to changes in the subject's capacity to perform a motor skill. To distinguish between lesion-produced reward deficits and operant motor/performance impairment, a more sophisticated behavioural technique, the rate-frequency curve-shift paradigm (Edmonds & Gallistel, 1974; Miliaressis, Rompré, Laviolette, & Coulombe, 1986; Stellar, Waraczynski, & Wong, 1988) is used in the design of modern self-stimulation lesion experiments. In this method, the experimenter varies the stimulation frequency from trial to trial, keeping the current constant within a session. The rate of lever pressing is then plotted against the various pulse frequencies, yielding a rate-frequency curve. In this paradigm, rewarding effectiveness is measured by the position

of the curve along the frequency axis. The curve's position is usually summarized by one statistic, namely the number of pulses required to sustain half of the maximum rate of responding, termed the "required number". If the required number is low, relatively few pulses are needed to sustain responding. If a lesion or other manipulation shifts the required number to higher values, then that suggests that the stimulation reward efficacy has been degraded, and thus more pulses are needed to sustain the previous level of responding. On the other hand, lesion-induced motor deficits, in essence similar to increases in task difficulty, are more likely to lower the asymptote of the rate-frequency curve, while leaving the lateral position of the curve little changed. The strength of the curve-shift paradigm lies in the potential it offers to dissociate two distinct effects of a physiological manipulation: performance capacity and stimulation effectiveness. Furthermore, this method is insensitive to the nonlinearity of the rate-frequency curve, a feature that can transform a small change in effectiveness into a large change in rate or transform a large change in effectiveness into a small (or unobservable) change in rate.

Although the curve-shift method has proved to be more reliable than simple performance measures, it is not devoid of interpretation difficulties. Edmonds and Gallistel's (1974) initial report on the concept of curve-shift analysis, employing the runway paradigm, was accompanied by a full

range of validation experiments. Decreasing stimulation efficacy such as lowering current intensity shifted the required number to higher values, while leaving the maximum rate of responding unaffected. In contrast, multiple performance manipulations such as administration of subparalytic doses of curare, increases in task difficulty, even haphazardous occurrence of disease, did not appreciably change the required number, at least in some subjects. The weakness of the study lies mainly in the inadequate quantitative resolution of the shifts allowed by the technology of the time. When performance capacity was challenged curves did not shift more than the interstimulus step of 0.3 $\log_{10}$ units. Especially because of all the evidence that drug intervention and lesions frequently produce lateral shifts of no more than 0.1-0,2 $\log_{10}$ units, the possibility remains that significant performance-induced changes in threshold might have been detected with a finer testing resolution. Nonetheless, a study by Miliaressis and Rompré (1987) showed that self-stimulating rats concomitantly stimulated through a second electrode in a motoric brain region performed as if the value of the reinforcing stimulation was not altered, despite reductions in asymptotic performance.

Further experiments have demonstrated that changes in self-stimulation thresholds cannot always be attributed to variations in reward. Fouriezos, Bielajew, and Pagotto

(1990) evaluated the effect of increasing task difficulty by weighting the lever on BSR thresholds. Self-stimulation rates and frequency thresholds were both affected by changes in response effort. Lateral shifts were found to be no more than 0.1 $\log_{10}$ units, but unfortunately testing was restricted to a limited range of task difficulty and was actually stopped when appreciable shifts became evident. It would have been of interest to follow the reward degradation at maximum efforts, using heavier weights, so as to see the range of influence that the task effort has on stimulation effectiveness.

Relevant to any discussion of the quantification of reward and performance effects is the notion of the dynamic interval of the reward curve. The dynamic interval is defined by the difference between the pulse numbers at which the rising portion of the rate-frequency curve begins and ends. Notwithstanding any rightward shifts, alterations of rate-frequency functions due to performance factors include depression of the asymptote and, often, changes in the slope of the rising curve. If there is a scalar transformation of the function whereby performance is reduced by the same proportion at all levels, then the dynamic interval of the curve is left unchanged. On the other hand, nonscalar transformations may result in dynamic interval changes. The essence of this discussion is that increases in the dynamic interval result in displacement of the frequency required to

support half-maximal responding towards higher values along the abscissa without necessarily producing a lateral shift of the entire rate-frequency curve.

Miliaressis, Rompré, Laviolette, and Coulombe (1986) assessed the behaviour of the rate-frequency curve in relation to both task difficulty and pharmacological manipulations. They observed deviations in thresholds, up to 0.2 $\log_{10}$ units, after performance manipulations, that were accompanied by decreases in maximum rates and increases in the dynamic interval. These findings offer some food for thought for those interested in the correct interpretation of lesion results. Small shifts can be considered meaningful only if they are not accompanied by any depression in the corresponding asymptotic rates and/or any changes in the slope of the rising curve.

Counter model: quantification of the lesioned substrate

Is there a way to link the quantitative information that rate-frequency functions provide about postlesion changes in reward effectiveness, with the degree of damage suffered by the directly activated substrate? An answer to this question is offered by the counter model of spatial and temporal integration of the output of the first stage neurones. Trading off pulse frequency, a temporal variable pertaining to the number of times a neurone fires, and current, a

spatial variable pertaining to the number of neurones fired by the stimulation, it has been shown that the required values of two parameters are often inversely proportionate. The linearity of current versus the inverse of the frequency function indicates that the number of action potentials fired by the first order neurones determines the rewarding effect of the stimulation, regardless to the spatiotemporal distribution of impulses within a time "window" of fixed duration. If the stimulating electrode had an infinitely small tip and was located within a bundle of reward fibres, the counter model could be reduced to two assumptions. First, the number of stimulated axons is proportionate to the current and second, the number of action potentials carried by the first-stage neurones is the product of the number of axons fired by the stimulation and the number of pulses in the stimulus train. Thus, since the processes that determine the reward experience perform as a counter of action potentials, it is as if the rewarding effect produced by, say, 400 fibres each carrying one impulse is the same as that produced by 200 fibres each carrying two impulses.

Under the assumption that the outputs of all the directly stimulated fibres are weighted equally, or vary randomly in weight as a function of the location of the fibres, the above theoretical considerations allow experimenters who use lesions to view the percentage change in magnitude of the lateral shifts of the rate-frequency

function as an index of the proportion of destruction of the reward relevant neurones. To illustrate from the previous example, if a lesion were to destroy 50% of the 400 reward neurones the required number would have to increase by 0.3 $\log_{10}$ units ($400/200 = 2 = 10^{0.3}$), and the fibres would now have to carry two action potentials instead of one in order to produce the same total number of action potentials and the same behavioural effect as that produced before the lesion. Similarly, a 0.1 $\log_{10}$ unit shift can be considered equivalent to 20% destruction of the substrate (example pertaining to the 400 fibre substrate: $10^{0.1} = 1.25$, thus, number of lesioned reward fibres is given by $(400 - 400/1.25)$ which equals to 80 fibres, or 20% of the substrate). Note that the assumption behind these calculations is that the lesion has actually damaged first-order neurones and not modulatory or efferent pathways to these neurones.

It is probably worth mentioning, in this context, a concern stemming from the notion of modulatory transmission in the reward pathway. Decreases in stimulation efficacy following lesions cannot be linked to any one stage of the system, because stimulation-induced behaviour can be affected by damaging neurones other than the first-order ones. These could include modulatory, perhaps inhibitory, neurones gating the pathway carrying the reward signal, or even neurones efferent, several synapses away, to the ones directly

activated by the electrode.

Previous lesion studies employing the curve-shift method

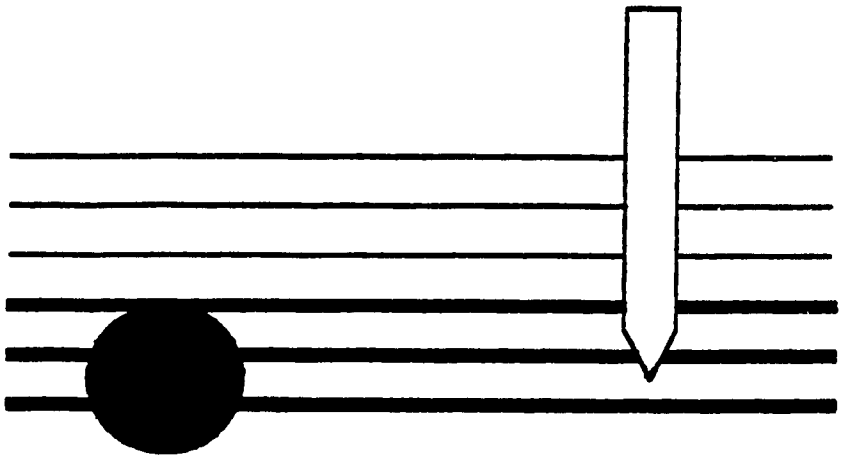
The scientific literature on BSR lesion studies is ample, yet only a handful of those experiments were conducted employing behavioural measures, such as the curve-shift paradigm, that could be interpreted unambiguously. Failure to demonstrate large postlesion decreases in reward effectiveness has been the pattern in much of the recent work. For example, electrolytic lesions of different regions of the amygdala (Waraczynski, Ng Cheong Ton, & Shizgal, 1990), and dorsomedial hypothalamus (Waraczynski, Conover, & Shizgal, 1992) have not resulted in marked changes in the rewarding effectiveness of MFB stimulation. Knifecuts placed in the septum have also failed to produce substantial increases in the frequency threshold for LH stimulation (Waraczynski, 1988). However, the most promising results in Waraczynski's study pertain to the transection of the lateral preoptic area (LPOA) which resulted in increased thresholds in some subjects. Likewise, Janas and Stellar (1987) found rightward shifts in the rate-frequency curve after knifecuts that transected the caudal portion of the LPOA.

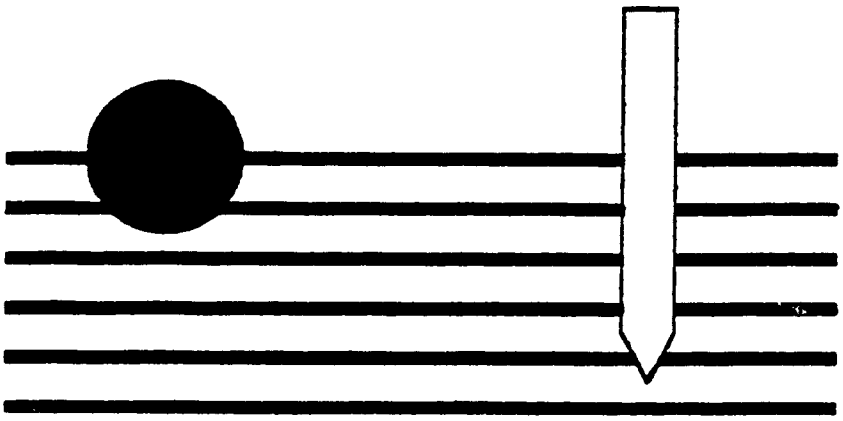
There is now direct evidence that self-stimulation of the LH and the VTA can be diminished by making lesions at the border of the LH and the LPOA. Of the seven well defined

electrolytic lesions that damaged the antero-lateral MFB in Murray and Shizgal's study (1991), five were effective in displacing the rate-frequency functions for the LH and/or VTA sites toward higher frequencies. All five effective lesions were located near the junction of the LPOA and the LH, whereas both of the ineffective lesions impinged on more medial parts of the LPOA. In one of their subjects for which rate-frequency curves were collected at two currents, the lesion produced an effect only at one of the currents.

Testing at only one current may have contributed to the failure of many of the lesion studies in identifying reward-relevant cellular groups. The *ad hoc* uncertainty about the relative position of the lesion with respect to the stimulated substrate, renders testing at three currents a useful adaptation of the curve-shift paradigm, developed to discern misalignments between activated neurones and the lesion (Fig. 1). Stimulation at successively higher currents results in the activation of successively greater number of relevant fibres. In a situation where the stimulating electrode is perfectly aligned with an effective lesion low currents are expected to bring about shifts in the rate-frequency curves, whereas in the case of misalignment, the spatial discrepancy between the electrode tip and the lesioned neurones renders only high currents effective in showing lesion-induced deficits in BSR. Murray (unpublished doctoral dissertation) extended the findings pertaining to

Fig. 1. Effect of current on lesion and electrode alignment. In the top diagram, the lesion is aligned with the electrode tip. The largest increase in the magnitude of the lesion effect, is likely to be observed at the low current, which recruits fibres running near the tip of the electrode. In the bottom diagram, the trajectory of the lesioned fibres passes far from the electrode tip. The largest increase in the magnitude of the lesion effect is likely to be observed at the high current which recruits additional fibres at some distance from the electrode tip. Bold lines represent stimulated axons and the filled circle indicates the lesion.





the contribution of neurones in the basal forebrain in BSR by making electrolytic lesions at the anterior LH (ALH) using the three-current design throughout the experiment. In 5 out of the 14 subjects frequency thresholds were increased. Collision-like effects have been reported for stimulation sites in the anterior MFB between the LPOA and the ALH (Bielajew, Thrasher, & Fouriezos, 1987) and more recently Murray and Shizgal (1992) showed by means of the collision test, that reward-relevant neurones directly link the ALH and the VTA. Taken together, these data bear on the descending path hypothesis of MFB self-stimulation and reveal a principal candidate structure. However, they fail to specify whether the destruction of cell bodies or of fibres of passage coursing through this structure results in the observed changes in reward. Neurotoxins that kill neurones by a hyperexcitatory action mediated by glutamate receptors might make it possible to distinguish between these two possibilities.

Excitotoxins as a cell-body selective lesioning tool

Glutamate is the major excitatory transmitter in the brain and glutamate receptors are abundant throughout the CNS (Monaghan, Bridges, & Cotman, 1989). Endogenous glutamate and other excitatory acidic amino acids are thought to elicit synaptic responses mediated by at least three ionotropic receptor subtypes: N-methyl-D-aspartic acid (NMDA), kainate,

and α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA). There is an additional subtype that is a metabotropic receptor, whose activation results in second-messenger mediated actions. The glutamate receptor system is thought to be involved in many normal functions including learning (Morris, 1989), but has also been implicated in a number of degenerative diseases as well as in the neuronal cell death that takes place after insults to the brain, such as head trauma and stroke (Olney, 1989). Abnormally high levels of stimulation of the ion-channel-linked receptors, through prolonged electrical stimulation (Sloviter, 1983) or epileptiform activity (Furshpan & Potter, 1989), trigger a destructive cascade of events that can massively kill neurones. Furthermore, it has long been known that systemic administration of glutamate destroys neurones in certain regions of the brain (Olney, 1969b). Glutamate neurotoxicity becomes an attractive means of selectively lesioning particular brain structures. When injected into the CNS, structural analogs of excitatory amino acids, known as excitotoxins, have the property of selectively destroying cell bodies while sparing axons afferent to or passing through the lesioned area.

The precise mechanism of neurotoxicity is still unresolved. Although all three ion-channel-linked receptors mentioned above are capable of mediating toxicity alone (Frandsen, Drejer, & Schousboe, 1989), the most studied of

these is the NMDA receptor. A particular feature of the NMDA receptor, in addition to its well-known associative activation involving both chemical and voltage gating, is its role in controlling permeability to calcium. Calcium entry into the cell mediates the destructive effect of excitotoxins, and evidence indicates that it is mainly through NMDA channels that toxic calcium entry occurs (Choi, 1987). Sustained disruption of intracellular calcium homeostasis, initially resulting from enhanced influx of extracellular calcium and potentially magnified from impairment of calcium extrusion and calcium release from intracellular stores, leads to the activation of cytotoxic mechanisms and to subsequent loss of cell viability. Impairment of mitochondrial function, disruption of cytoskeletal organization, proteolysis, membrane degradation, and chromatin fragmentation, are all dependent on calcium-activated enzymatic processes (Nicotera, & Orrenius, 1992).

Since the bulk of glutamate receptors reside in the dendrites and somata, fibres are not affected by excitotoxins unless the somata from which they originate degenerate. Indeed, release of neurotransmitter from axons passing through excitotoxic lesions is normal (Hastings, Winn, & Dunnett, 1985), and anatomical tracers such as horseradish peroxidase show normal transport through the lesion (Schwarcz, Hokfelt, Fuxe, Jonsson, Goldstein, & Terenius, 1979). Nonetheless, demyelination, perhaps caused

nonspecifically by local inflammation, accompanies cell death (Coffey, Perry, Allen, Sinden, & Rawlins, 1988), thus altering the conduction properties of fibre systems coursing through the area of injection. Inflammatory myelin breakdown is seen in the presence of infiltrated immune cells such as macrophages which presumably are recruited from the bloodstream into the lesioned area to phagocytose necrotic debris. Microglia, abundantly present in the glial scar formed in the vicinity of the lesion, contribute to brain inflammation by engulfing debris and by releasing cytotoxins, growth factors and immunomodulators (Giulian, 1987).

Demyelination complicates the interpretation of lesion experiments since changes in reward can be attributed to ill-conducting axons of passage as well as to cell body loss. Nerve impulses are conducted in demyelinated axons with reduced velocity, and conduction block has been demonstrated in central demyelinated fibres (Waxman, 1977). Despite the caveats of working with a less perfect lesioning tool than originally claimed, there is a way to circumvent or diminish the problem of nonspecific damage. A study conducted by Stellar, Hall, and Waraczynski (1991) showed the demyelination zone to be smaller than the zone of neural killing. This finding offers the possibility of selective cell body lesioning in the area of interest by injecting the excitotoxin further away from the targeted area. While there would still be an area of demyelination, it might be located

in a region of relatively few fibres of interest.

Considerations involving the problem of demyelination along with the application of improper behavioural measurement techniques render previous excitotoxin studies of MFB self-stimulation difficult to interpret. For example, the effect of destruction of intrinsic neurones of the LH (Lestang, Cardo, Roy, & Velley,1985; Nassif, Cardo, Libersat, & Velley, 1985; Velley, 1986; Sprick, Munoz, & Huston, 1985) and the LPOA (Huston, Keifer, Buscher, & Munoz, 1987) by excitotoxins remains unclear due to several methodological problems. Proper threshold measures and the evaluation of demyelination were both implemented by Stellar et al.(1991) assessing the effects of excitotoxin-mediated cell damage near the LH stimulating electrode. The conclusion of this study was that neurones intrinsic to the LH do not play a major role in the rewarding effect of LH stimulation; large increases in required number were only seen when demyelination extended to the electrode tip. However, in the absence of demyelination at the electrode tip, increases in the frequency threshold around 0.1 $\log_{10}$ units were observed for some of the rats. This finding along with conclusions from Murray and Shizgal's (1991) study suggest that further investigation of the anterior MFB/basal forebrain as a putative first-order neurone containing region in the CNS is warranted.

Aim of the present study

The present study takes advantage of the relative selectivity of excitotoxin lesions to investigate whether basal forebrain structures, in particular the LH/LPOA border, contains at least some of the long-sought cell bodies that give rise to the first-order components of the BSR substrate. To have better control over the manipulated variables in this experiment, NMDA, a selective agonist to its calcium-permeable receptor whose mode of action is better understood than that of other neurotoxins, was chosen to be the excitotoxin used in this study. Additional advantages of NMDA are its decreased likelihood to cause damage into remote regions as compared, for example, to kainate (Hastings et al., 1985) and its low cost. Furthermore, Stellar et al.(1991) have found that NMDA produces less demyelination than ibotenic acid. Stimulating electrodes were implanted at self-stimulation sites in the LH and VTA and a cannula was aimed so as to position the area of cell body loss after the injection of NMDA at sites similar to those lesioned electrolytically by Murray and Shizgal (1991). Changes in the reward magnitude of the stimulation were inferred from lateral displacements of rate-frequency curves obtained at three different currents at each site.

Method

Subjects

Eighteen male rats of the Long-Evans strain weighing 400-550 grams at the time of surgery served as subjects. They were individually housed in plastic cages with ad lib access to food and water and were kept in a temperature controlled environment with a reverse 12 hour dark/light cycle. Behavioural testing was restricted to the dark phase of the cycle.

Surgery

Surgery was performed under Somnotol anaesthesia (75 mg/kg i.p.) with supplements administered as required. To reduce bronchial secretions, Atropine sulphate (0.5 mg/kg) was injected subcutaneously 20 min prior to anaesthesia. After the induction of deep anaesthesia, assessed by a lack of corneal and tail-pinch reflexes, subjects were secured in a stereotaxic instrument with the skull levelled. Following craniotomy, monopolar electrodes were aimed at the ipsilateral LH (2.8 mm behind bregma, 1.7 mm lateral from the midsagittal sinus, and 7.8 mm below the dura matter) and VTA (4.8 mm behind bregma, 0.9 mm from the midsagittal sinus, and 7.7 mm below the dura matter). The electrodes were constructed from 00 insect pins insulated with Formvar to

within 0.5 mm of the tip. Four jewellers screws were embedded in the cranium to serve as anchors for the assembly. A wire wrapped around one of the four screws served as the anode. Each rat was also implanted with an ipsilateral stainless steel 24 gauge cannula (15 mm in length), which later served as a guide for the intracerebral placement of the 1.0 µl Hamilton microsyringe used to inject the excitotoxin. Throughout testing, a 20 gauge obturator extending up to the injection site blocked the cannula. Stereotaxic coordinates for the cannulae varied across rats (0.8 - 1.8 mm behind bregma, 2.0 - 2.5 mm from the midsagittal sinus, and 3.0 - 6.5 mm below the dura matter). At the conclusion of the surgery, male amphenol pins attached to copper wires soldered to the electrodes and the ground wire were inserted into a nine-pin connector which was securely cemented onto the rat's head with dental acrylic. After each surgery the rats were injected with morphine (5.0 mg/kg i.p.) to reduce pain, and a topical antibiotic was applied to the incision to prevent infection. Animals were allowed to recover for at least five days before the start of behavioural testing.

Following the collection of baseline self-stimulation data, rats were anaesthetised and standard stereotaxic procedures were used as previously described. The obturator was removed from the cannula, and a stereotaxically mounted Hamilton microsyringe was guided into the injection site and

then left there for 5 min. Sixteen rats received 0.5 µl 0.14 M (70 nmoles) NMDA (Sigma) dissolved in modified Ringer's solution (Moghaddam & Bunney, 1989) and pH adjusted to 7.1 - 7.4 with NaOH. The other three rats received 0.5 µl vehicle alone. NMDA was delivered by a "step-by-step" infusion of 0.05 µl every 60 s. After injection of the total dose of NMDA or vehicle, the syringe was left in place for another 5 min to allow for diffusion.

Behavioural testing

Training apparatus

Before actual testing, subjects were screened for self-stimulation in wooden boxes (25 cm x 25 cm x 70 cm) with Plexiglas front panels and wire-mesh floors. A Lehigh Valley lever protruded from the centre of one wall, 5 cm above the floor. A yellow key light signalling stimulus availability was located 5 cm above the lever. A 7-channel, slip-ring commutator provided a connection to the stimulator, while allowing the subject to circle without twisting the leads. Electrical stimulation was provided by dual constant-current amplifiers (Mundl, 1980) and controlled by hand-set integrated circuit pulse generators. It consisted of fixed, 0.5 s trains of cathodal, rectangular pulses, 0.1 ms in duration. Stimulation parameters were monitored on an

oscilloscope. To prevent tissue damage from electrode polarization, the stimulator outputs were connected electronically, between stimulation pulses via a low impedance path.

Training procedure

A stimulation cable was screwed to the 9-pin connector on the rat's head. The rat was transferred to the testing cage and the cable was connected to the commutator. Low levels of noncontingent stimulation were delivered to either stimulating electrodes. Testing was terminated if the rat manifested signs of aversion (e.g. vocalisation, freezing), severe,response disrupting, motoric effects or disinterest; otherwise, subjects were trained to self-stimulate. A typical training sequence consisted of several steps. Once rats learned to perform on a continuous reinforcement schedule, they were shaped to respond only when the key light was turned on. To enhance responding, prior to the illumination of the light signalling availability of stimulation, 5 noncontingent trains of priming stimulation were delivered with the same parameters that were to serve as the reward.

As a preparation for subsequent testing, the animals were trained using different currents and frequencies to determine the range of parameters that sustained self-

stimulation. Extinction trials were interspersed with self-stimulation ones so that subjects would reliably return to the lever after priming stimulation of sufficient strength.

Testing apparatus

Actual testing was conducted in a computer-controlled setup housed in a dedicated testing room. Test chambers were similar to those used for training but constructed entirely from Plexiglas. They were equipped with removable floors, two levers located on opposite walls 5 cm from the floor and 5 cm from the nearest corner, a white houselight (40 watt), and two key lights situated 3 cm above each lever, a yellow one on one side and a red one (not used in this study) on the other. The test chambers were enclosed in 50 cm x 50 cm x 90 cm sound-attenuating boxes. The Styrofoam insulation along with the background noise from ventilation fans at the back of the boxes prevented external sources of noise from disrupting the rats during testing. Rats were monitored by means of a remote-controlled video camera from an adjoining control room.

Typically, the electrical stimulation parameters used for testing were the same as those used during training. In some subjects adjusting those parameters was required to minimize certain stimulation-induced side-effects such as seizures. This was usually accomplished by shortening the

train duration and interposing a brief fixed-interval delay. Stimulation trains were monitored on an oscilloscope.

Testing procedure

A testing session consisted of several 30 s trials. Each trial began with the houselight turning off for 0.5 s, followed by 5 trains of priming stimulation identical to the stimulation that would serve as the reward. Availability of the stimulation was signalled by the yellow key light being illuminated. During a daily session, four rate-frequency curves were collected at each of three currents for each stimulation site. The first curve at each current was considered a warm-up and was not used in the data analysis. The stimulation frequency for the first trials was chosen so as to maintain asymptotic (maximal) responding. On subsequent trials, the frequency was decreased in 0.05 $\log_{10}$ units steps until the rat emitted fewer responses than the criterion for quitting, set by the experimenter, on two consecutive trials. Quitting was typically defined as 10 responses, but ranged up to 5 responses above or below this value for rats that either showed erratic extinction or had low maximum rates. Animals that self-stimulated on both LH and VTA electrodes were tested twice per day, with one session devoted to each stimulation site.

The number of pulses required to sustain half-maximal

rate of responding (required number) was calculated for each rate-frequency curve. This datum is considered indicative of the position of a curve along the x-axis. The required number obtained from the three rate-frequency curves at each current were then averaged. Thus, for each stimulation site, estimates of the mean and standard error of the mean of the required number, one for every current, were obtained daily. Conclusions pertaining to the effectiveness of lesions were drawn from plotting the pre- and post-lesion mean required number for each test session as a function of days, at each of the three tested currents.

Maximum response rates were obtained by choosing, from individual rate-frequency curves, the highest number of lever-presses. These data were then averaged for each current level, yielding a mean maximum response rate for each current in each session.

In order to assess the effects of the lesions on the range over which the rate-frequency functions rose (dynamic interval), rate-frequency curves were fit with a broken-line function (Gallistel & Freyd, 1987), composed of a straight line connecting a lower and an upper asymptote. The dynamic interval was calculated from the difference between the upper and the lower break-points.

Statistical analysis

To give a rough idea of the statistical significance of any postlesion shifts, 95% confidence intervals were constructed around the mean of the required number of pulses, the mean of the maximum response rates, and the mean of the dynamic range obtained for the five days preceding the lesion. The standard deviation of the 5 means was used as an estimate of the standard error of the mean (Ferguson and Takane, 1989, p.163). Confidence intervals were obtained by multiplying the standard deviation of the 5 baseline means by the t value associated with $p = 0.05$ level of significance for 4 degrees of freedom ($t = 2.776$) (Waraczynski, 1988). Because postlesion physiological changes are likely to be time-dependent, data obtained on every postlesion day were compared individually to the confidence intervals instead of being grouped. In the case of the dynamic range analysis, data obtained on five randomly selected postlesion sessions at each current were individually compared, at each current, to the obtained confidence intervals.

Histology.

At the end of the experiment, each rat was killed, under deep Somnotol anaesthesia, by exsanguination. The circulatory system was perfused with heparinized, phosphate buffered saline followed with 10% formalin. The brain was

removed and soaked in 10% formalin for at least two days and was transferred to 20% sucrose formalin solution one day prior to slicing. The tissue was frozen with dry ice onto a microtome stage, then was placed in a freezing microtome and allowed to equilibrate at -20 °C for 1.0 hour. Two or three adjacent sagittal sections, 30 µm thick, were obtained at intervals not exceeding 0.15 mm, starting from about 1.0 mm lateral to the injection site. The sections were placed onto either gelatin-coated or commercially available, electrostatically treated slides and were allowed to dry for a minimum of one day. Formol-thionine and hematoxylin (Weil procedure) stains, specific for cell bodies and myelin, respectively were used to assess electrode tip location and the extent of lesion damage. The area affected by the NMDA was determined by microscopic comparison of tissue appearance in lesioned versus nontreated brain slices. Bleaching in hematoxylin stained tissue and accompanying disappearance of stained fibres were taken as evidence for demyelination. Starting at low magnification, the extent of cell loss and demyelination in relation to known brain landmarks was assessed. High magnification was used to visualize fine detail of lesion and demyelination boundaries. These, and the location of the electrode tips were recorded on sagittal plates from the Paxinos and Watson (1986) atlas.

Results

Rationale and format of data presentation

Plots of the required number of pulses as a function of time pre and postlesion are presented for all subjects in Figs. 2-5, 16-21, and 30-34. Error bars represent the standard error of the mean for the corresponding test day. The parallel dotted lines in each graph represent the mean of the required number of the five baseline days for each of the three currents. The extent of NMDA-induced damage as well as electrode tip locations are reconstructed onto tracings of sagittal plates from the Paxinos and Watson atlas (1986) in Figs. 13-15, 26-29, and 35.

For the purpose of data presentation, subjects are divided into three groups on the basis of the magnitude of the degradation of MFB reward. In the first group (group A, Figs. 2-5), NMDA lesions produced long-lasting increases in the required number that were sustained at a level of roughly 0.2 $\log_{10}$ units for at least one of the three currents at a stimulation site. The second group (group B, Figs. 16-21) consists of rats with immediate postlesion increases in the required number of at least 0.1 $\log_{10}$ units that either remained around that level throughout testing, or gradually recovered towards baseline levels. Indeed, the transient threshold increases are characteristic of the results for

this group. In the third group of rats (Group C, Figs. 30-34), lesions (Group C1) or injection of the vehicle (Group C2) produced no effect. The 0.1 $\log_{10}$ units criterion is a reasonable way to classify lesion effects on empirical grounds. In all cases, the 0.1 $\log_{10}$ units criterion exceeded the upper range of the 95% confidence intervals constructed around the mean of the five baseline days. Thus, the three groups A, B, and C classify respectively large, substantial, and no postlesion increases in the required number.

The assumption underlying the criteria for meaningful changes in stimulation reward effects, both large and substantial, is that asymptotic response rate remains unaffected after the lesion. To verify the validity of this assumption for the data at hand, averages for each session of the maximum response rates at each of three currents were plotted (in Figs. 6-8 and 22-25) as a function of time for all rats within groups A and B. Five prelesion testing days are shown along with the mean of the baseline data for each current, depicted by the dotted lines extending across each graph. Error bars are omitted to facilitate visual inspection of the graphs. Postlesion performance capacity was said to have decreased if the average maximum rate on three consecutive postlesion days was outside of the 95% confidence interval or was shifted by more than 0.10 $\log_{10}$ units, whichever was greater.

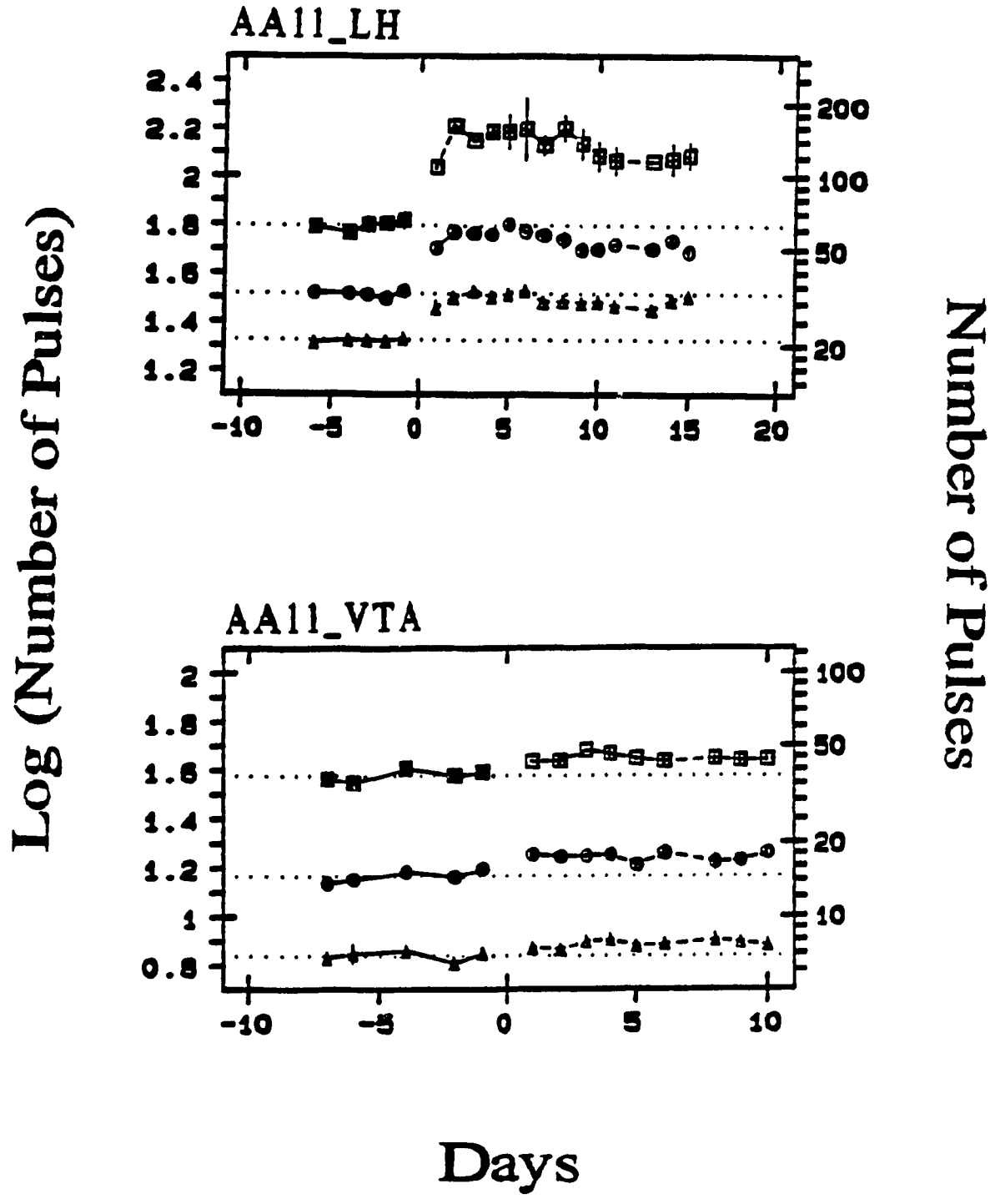
As mentioned in the introduction, when evaluating changes in required number that are accompanied by a decrease in response asymptote, it is important to look for changes in the dynamic range of the rate-frequency curves. Nonparallel curve shifts should not be interpreted as necessarily signifying reward degradation. In that light, for subjects in group A with changes in maximum rates, the dynamic ranges obtained on each of the randomly selected postlesion sessions at each current are tabulated and compared to the upper confidence limit around the mean of the dynamic ranges obtained for the five days preceding the lesion. For illustration purposes, the dynamic intervals of the five prelesion days and the five randomly chosen postlesion days are plotted as a function of time at each current in Figs. 9-12.

Group A: Large increases in the required number

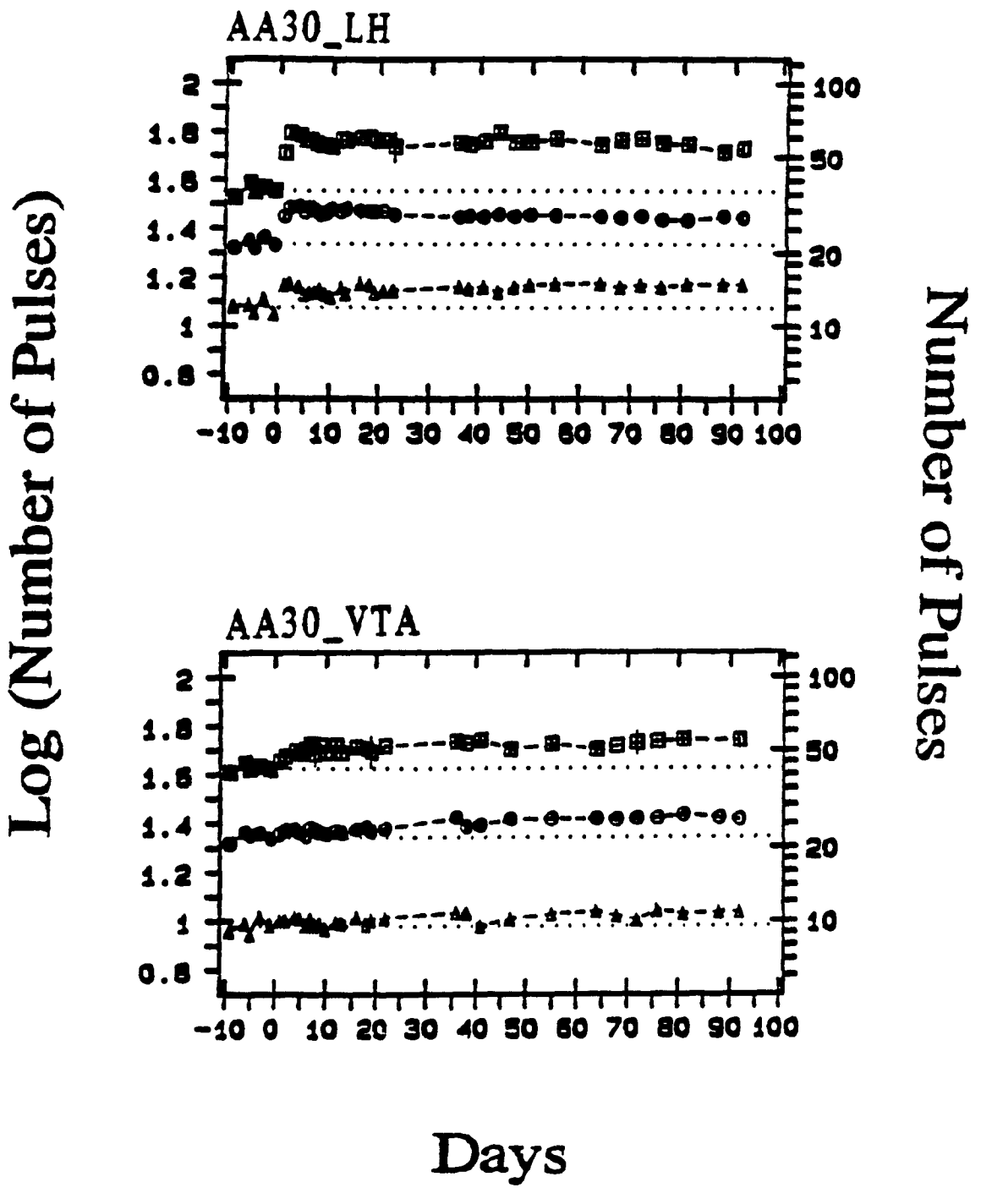
Group A comprises five rats, AA11, AA30, AA28, AA37, and AA39, only two of which (AA11 and AA30) were trained to lever-press for stimulation through both the LH and VTA electrodes. For the remaining rats, rate-frequency curves were collected for stimulation delivered to the LH site. Behavioural data for subjects in this group are shown in Figs. 2-5. Meaningful postlesion increases in the required number were only seen for stimulation delivered through the LH electrodes, although smaller increases were observed at

Figs. 2-5. Effects of lesion on self-stimulation in subjects AA11, AA30, AA28, AA37, and AA39 (Group A). The lesion in these subjects produced large increases in the required number. Each subject is identified in the top-left corner of the graph. Prelesion data are designated by negative values along the abscissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last prelesion test session. Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol. Squares represent values obtained at the lower current, circles represent values obtained at the middle current, and triangles represent values obtained at the higher current.

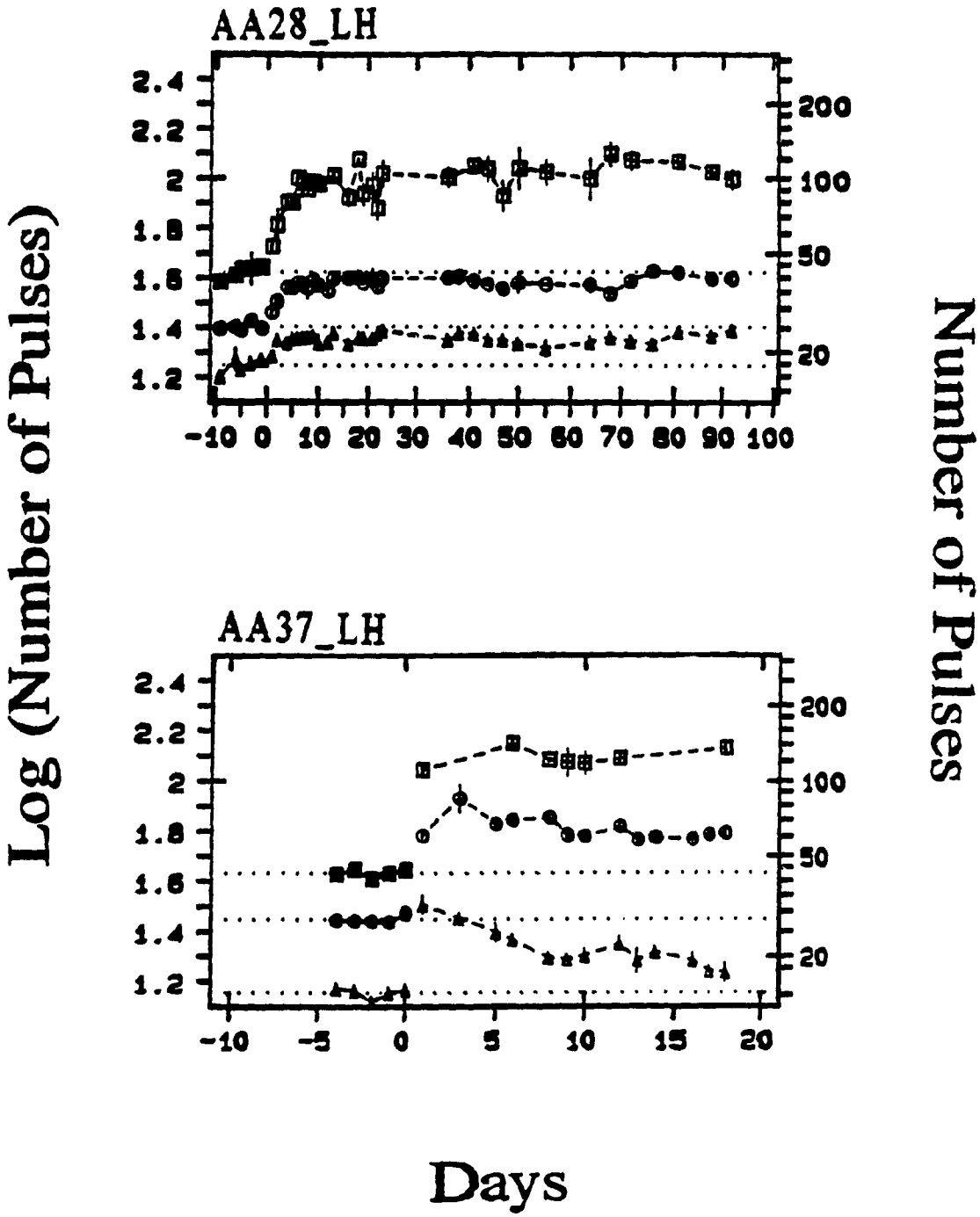
Group A

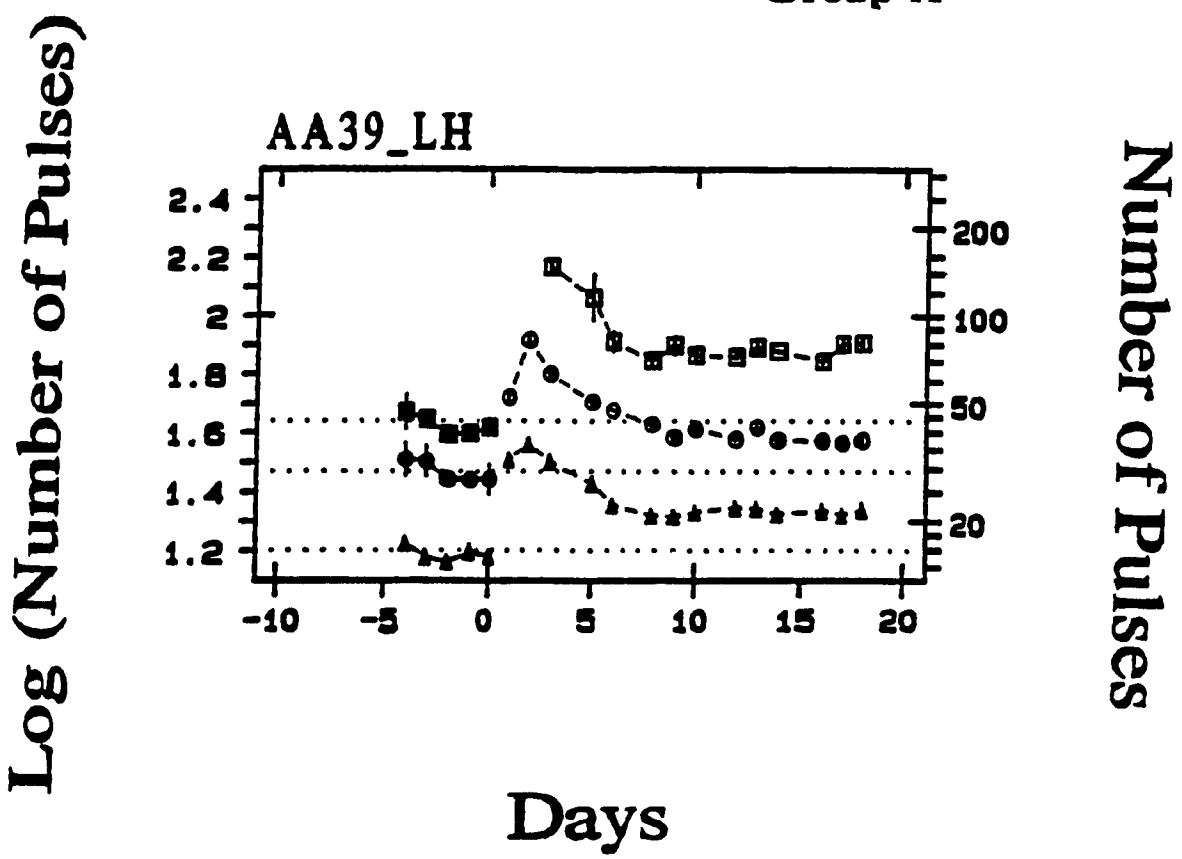

AA11_LH
2.4
2.2
2
1.8
1.6
1.4
1.2
200
100
50
20
-10
-5
0
5
10
15
20
AA11_VTA
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10
Log (Number of Pulses)
Number of Pulses
Days

Group A


AA30_LH
AA30_VTA
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10 0 10 20 30 40 50 60 70 80 90 100

Group A


AA28_LH
AA37_LH
Log (Number of Pulses)
Number of Pulses
Days
2.4
2.2
2
1.8
1.6
1.4
1.2
200
100
50
20
-10 0 10 20 30 40 50 60 70 80 90 100
-10 -5 0 5 10 15 20


Group A
AA39_LH
Log (Number of Pulses)
Number of Pulses
2.4
2.2
2
1.8
1.6
1.4
1.2
200
100
50
20
-10
-5
0
5
10
15
20
Days

the VTA sites. The reward efficacy of LH stimulation was most dramatically reduced in subjects AA11, AA28, and AA37, with the largest increases in required number, of about 0.4 $\log_{10}$ units above baseline, occurring at the lowest current. The threshold increase in the case of AA28 was gradual and stabilized after almost a week of postlesion testing.

For subjects AA30 and AA39, the required number for the lowest current stabilized at about 0.2 $\log_{10}$ units above baseline, although for AA39, there was a transient increase in threshold that reached approximately 0.6 $\log_{10}$ units from baseline, at the low current, shortly following the lesion. Stable shifts in the required number were also observed at the higher currents for all rats, ranging from approximately 0.1 $\log_{10}$ units to 0.3 $\log_{10}$ units at the middle current and from about .6 $\log_{10}$ units to 0.15 $\log_{10}$ units at the high currents. The exception occurred in the case of AA37, at the higher current, where an immediate increase in threshold was observed followed by a subsequent, gradual decrease towards baseline.

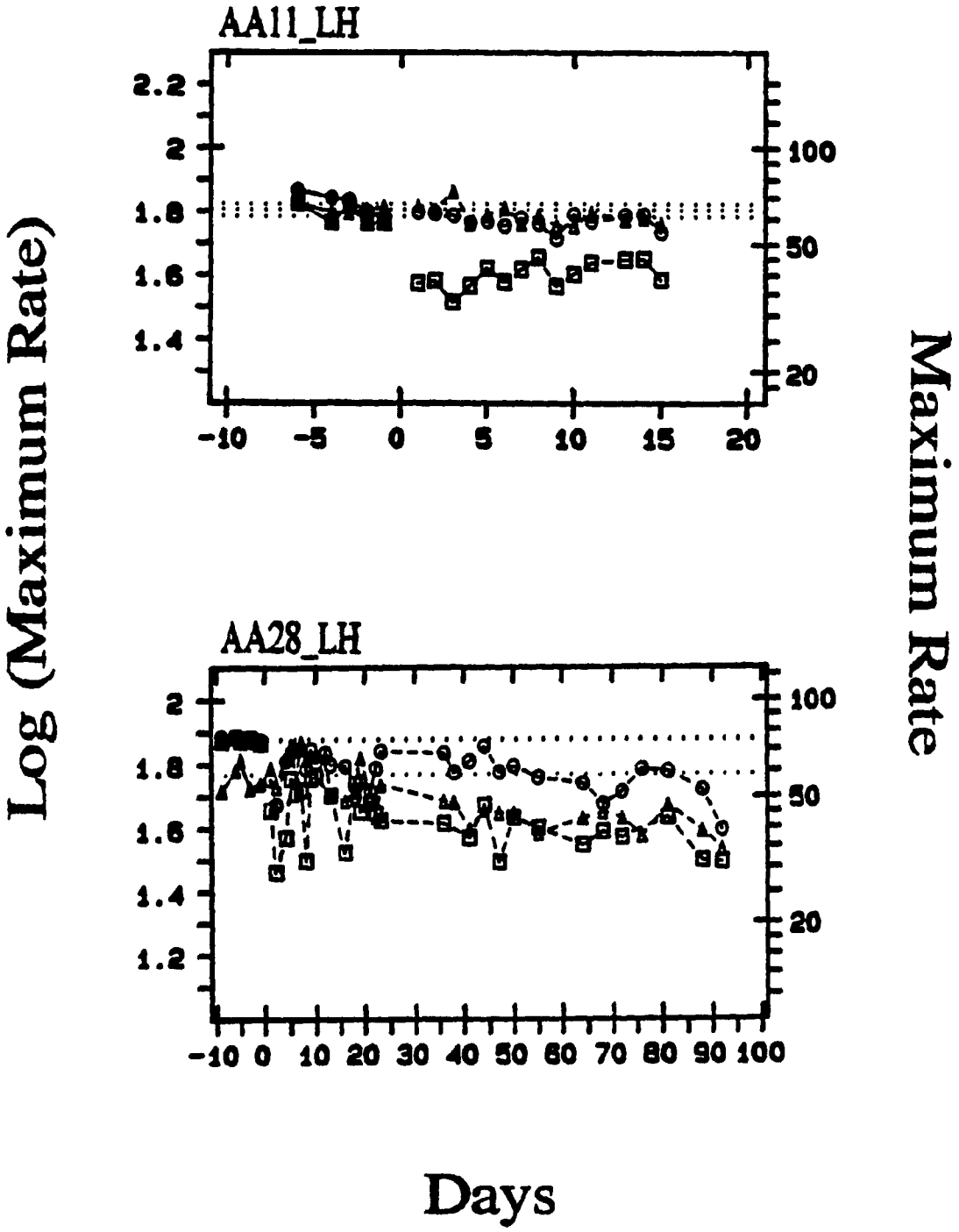
Postlesion testing was particularly prolonged for subjects AA28 and AA30. Remarkably, the elevated thresholds remained stable until the end of three month's postlesion testing. Given the data for these subjects, it is not unlikely to assume that the other effects observed would have

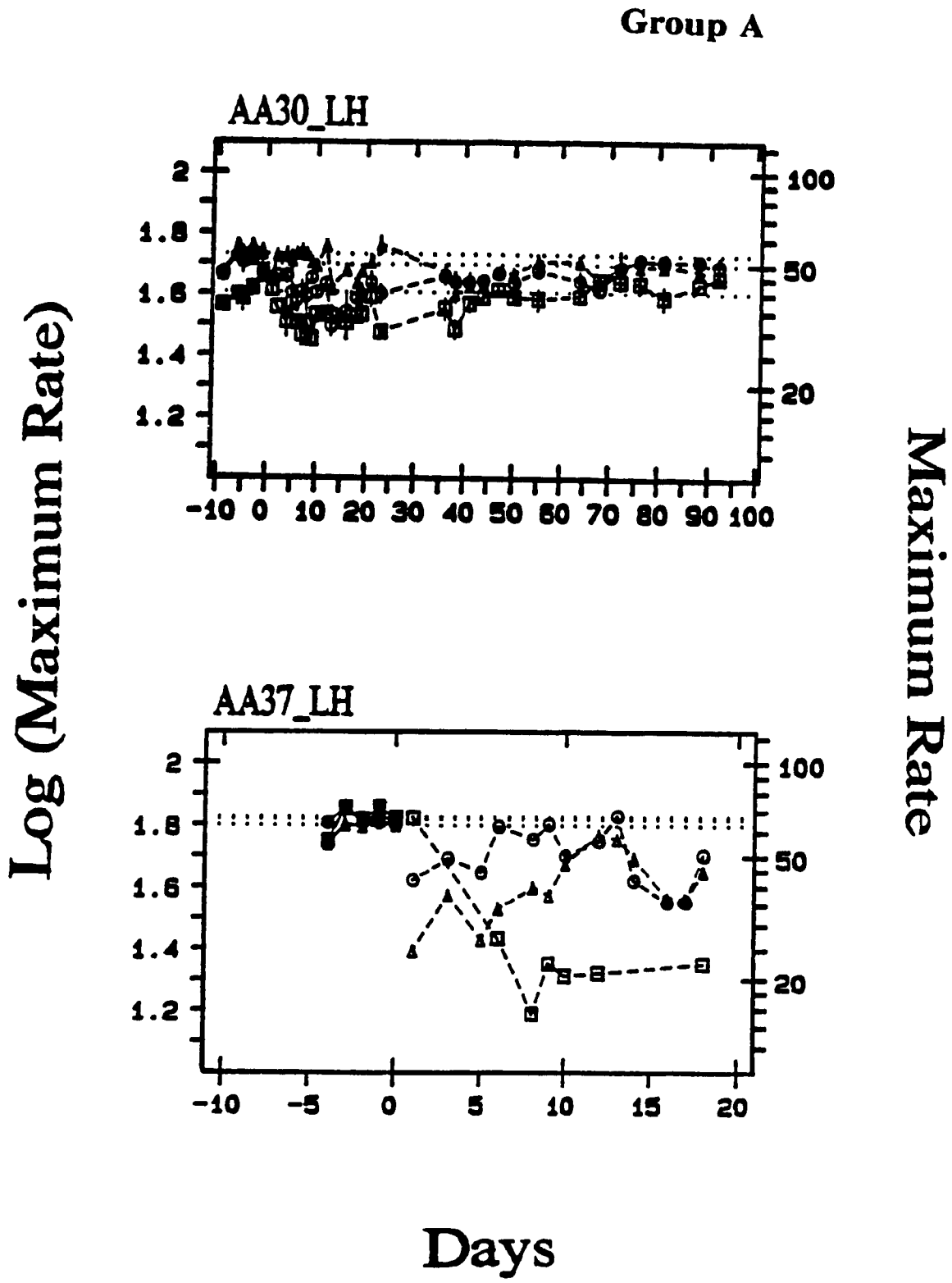
also remained stable over a long period of time.

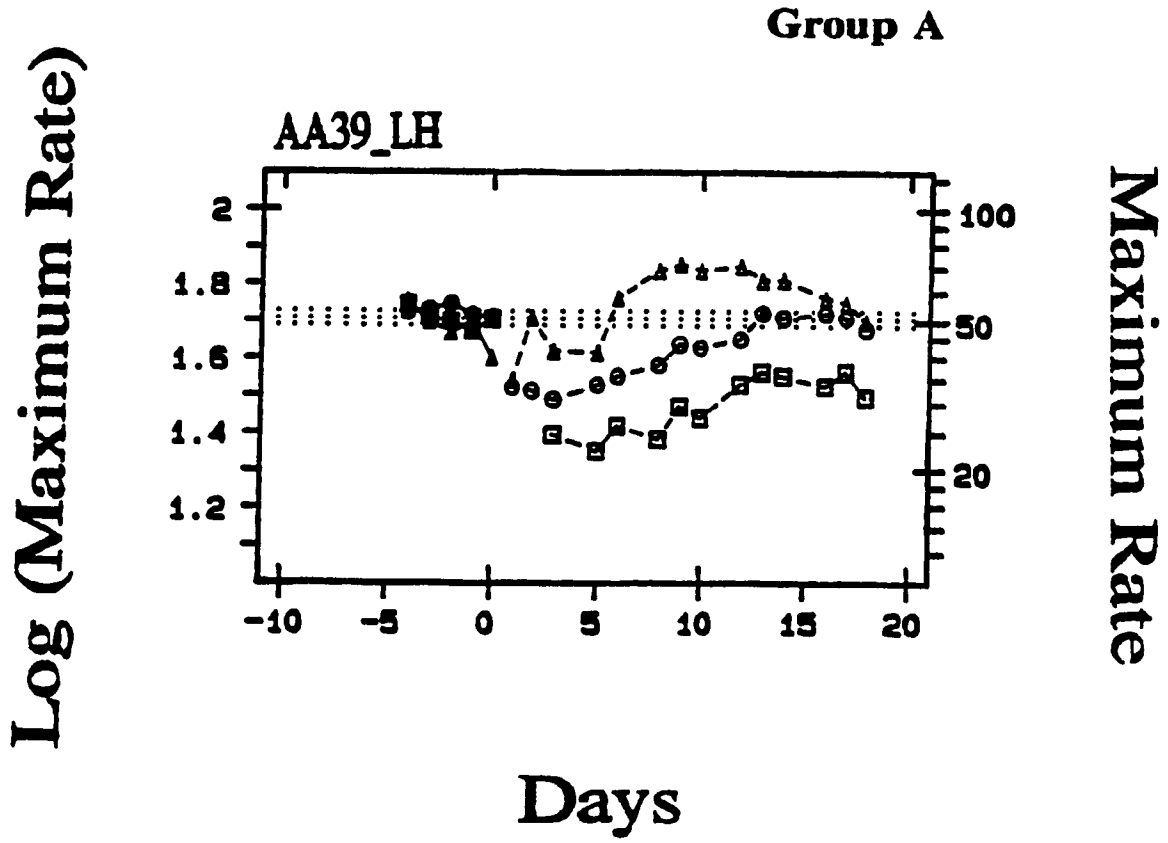
Maximum response rate graphs for subjects belonging in group A are shown in Figs. 6-8. Four of the five subjects (AA11, AA28, AA37 and AA39) with large increases in the required number also showed significant depression in maximum response rate. As was the case with the required number, the decreases in the maximum response rate were largest at the lower current. The largest decrease in the maximum response rate was seen in the case of subject AA37. At the lowest current, decreases reaching up to 0.6 $\log_{10}$ units below baseline were observed while at the middle and high current, response rates were variable and ranged from baseline to within 0.4 $\log_{10}$ units below baseline. It should be noted that the increases in the required number at the low and middle currents were approximately of the same magnitude, 0.4 and 0.35 $\log_{10}$ units respectively, but the decreases in response rates were predominantly observed at the low current. Similarly, AA11 showed a stable decrease in the maximum response rate only at the low current, of approximately 0.2 $\log_{10}$ units below baseline. The response rate at the middle current remained unchanged from baseline, in contrast to the required number for that current which increased by 0.3 $\log_{10}$ units. For subject AA39, significant decreases in the maximum response rate were evident at the low current, ranging from approximately 0.2-0.3 $\log_{10}$ units

Figs. 6-8. Maximum rates for subjects AA11, AA28, AA30, AA37, and AA39 (Group A). The lesion in these subjects produced large increases in the required number. Each subject is identified in the top-left corner of the graph. Prelesion data are designated by negative values along the abscissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last prelesion test session. Postlesion data are represented by open symbols. Error bars are omitted.

Group A


AA11_LH
2.2
2
1.8
1.6
1.4
100
50
20
-10 -5 0 5 10 15 20
AA28_LH
2
1.8
1.6
1.4
1.2
100
50
20
-10 0 10 20 30 40 50 60 70 80 90 100
Log (Maximum Rate)
Maximum Rate
Days


Group A
AA30_LH
2
1.8
1.6
1.4
1.2
100
50
20
-10 0 10 20 30 40 50 60 70 80 90 100
AA37_LH
2
1.8
1.6
1.4
1.2
100
50
20
-10 -5 0 5 10 15 20
Log (Maximum Rate)
Maximum Rate
Days


Group A
AA39_LH
Log (Maximum Rate)
Maximum Rate
2
1.8
1.6
1.4
1.2
100
50
20
-10
-5
0
5
10
15
20
Days

below baseline and at the middle current, peaking at 0.2 log10 units below baseline. No decreases were seen at the high current. For the middle current, the decrease in maximum rate was observed only for the first six postlesion sessions, a time course which coincides with the transient changes in the required number for this subject. Similarly, in the case of AA28, a substantial decrease in maximum response rate was observed at the low and middle currents. However, this decrease is observed beginning after two weeks of postlesion testing while the required number threshold remained stable.

Dynamic interval analysis was conducted for the four rats that showed decreases in the maximum rates. The results for individual rats are shown in Figs. 9-12. Each of these figures contains a table showing the average baseline dynamic interval, the upper 95% confidence limit, and the mean dynamic interval for each of the five randomly chosen postlesion sessions. A star indicates a significant difference between the postlesion data and the baseline mean. Accompanying this table will be a representative graph of the dynamic intervals as a function of time pre and postlesion.

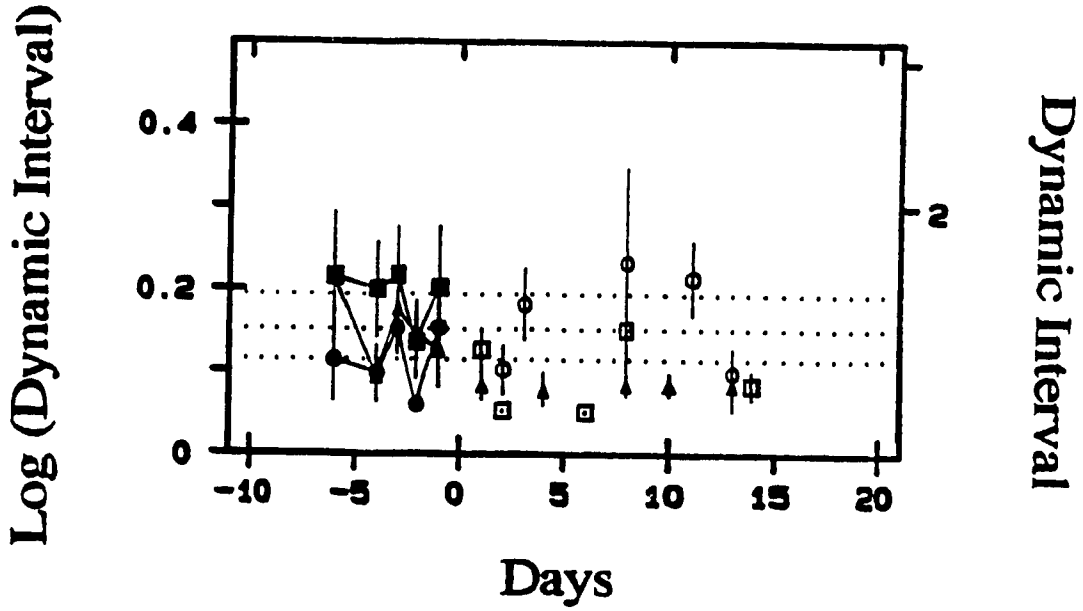
In general, no change was observed in the dynamic interval. Even in the case of AA37, which showed large decreases in response rate, the dynamic interval remained unaltered at the low and middle current. Paradoxically, the

Figs. 9-12. Dynamic intervals for subjects AA11, AA28, AA37, and AA39 (Group A). The table on the top shows the average baseline dynamic interval, the upper 95% confidence limit of the baseline dynamic interval, and the mean dynamic interval for each of the five randomly chosen postlesion sessions. A star indicates a significant difference between the dynamic interval of the postlesion sessions and the average baseline dynamic interval. The bottom graph shows the dynamic intervals for the baseline days and the five postlesion days as a function of time pre and postlesion. Prelesion data are designated by negative values along the abscissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last prelesion test session. Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

AA11_LH

Group A

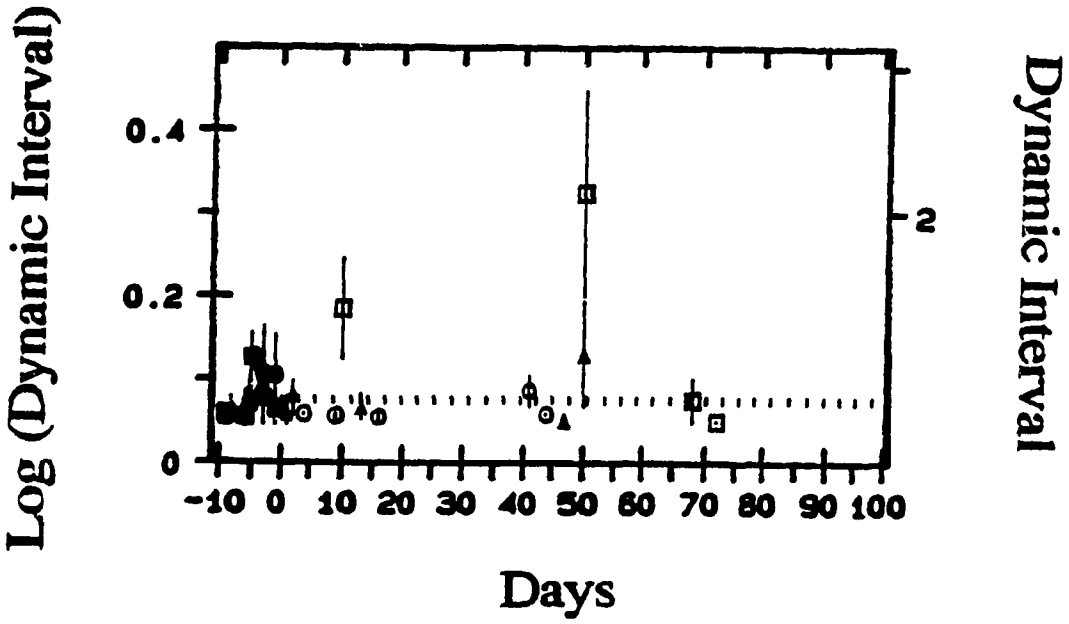
Current	Baseline Dynamic Range	Upper Confidence Limit	Sessions Postlesion				
			s1	s2	s6	s8	s13
LC	1.58	1.93	1.33	1.13	1.01	1.41	1.21
			s2	s3	s6	s11	s12
MC	1.30	1.68	1.26	1.13	1.70*	1.63	1.25
			s1	s4	s8	s10	s12
HC	1.41	1.90	1.21	1.20	1.21	1.21	1.21



AA28_LH

Group A

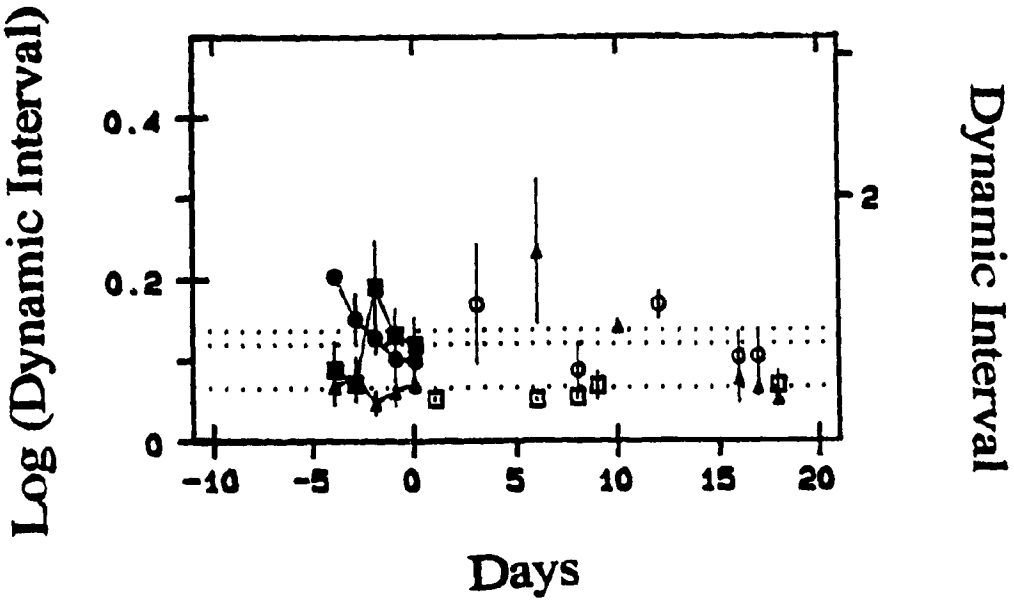
Current	Baseline Dynamic Range	Upper Confidence Limit	Sessions Postlesion				
			s1	s9	s23	s26	s27
LC	1.19	1.43	1.16	1.53*	2.11*	1.19	1.12
			s3	s8	s12	s20	s21
MC	1.20	1.41	1.14	1.14	1.13	1.22	1.14
			s1	s2	s11	s22	s23
HC	1.18	1.32	1.15	1.21	1.17	1.12	1.35*



Group A

AA37_LH

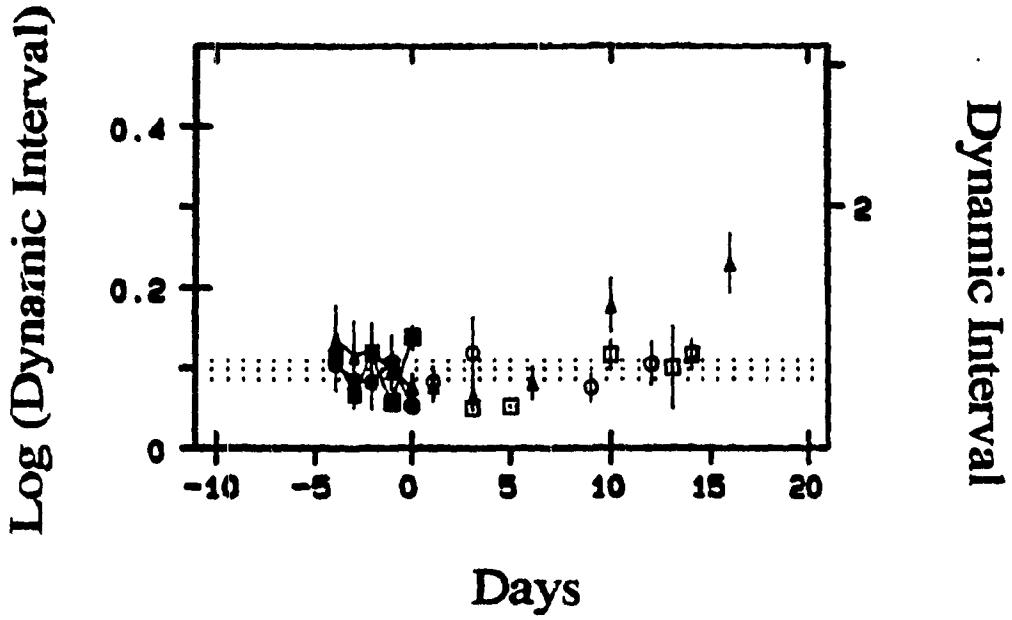
Current	Baseline Dynamic Range	Upper Confidence Limit	Sessions Postlesion				
			s1	s5	s6	s7	s12
LC	1.31	1.76	1. 12	1.12	1.13	1.17	1.16
			s2	s5	s8	s11	s12
MC	1.36	1.80	1.47	1.22	1.47	1.26	1.26
			s4	s7	s11	s12	s13
HC	1.16	1.25	1.71*	1.38*	1.19	1.16	1.13



AA39_LH

Group A

Current	Baseline Dynamic Range	Upper Confidence Limit	Sessions Postlesion				
			s3	s4	s6	s10	s11
LC	1.25	1.57	1.33	1.13	1.01	1.41	1.21
			s1	s3	s7	s9	s11
MC	1.22	1.40	1.21	1.31	1.19	1.27	1.31
			s1	s3	s5	s8	s12
HC	1.28	1.50	1.20	1.16	1.02	1.51*	1.70*

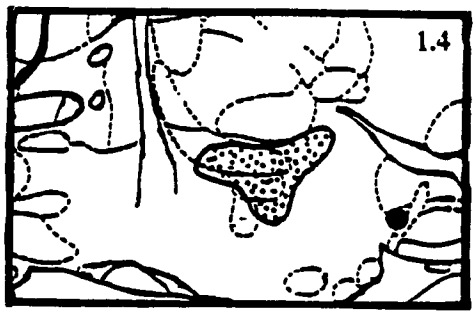


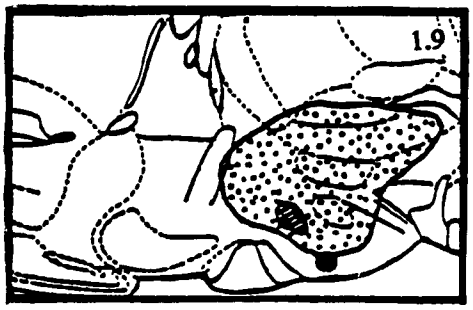
only increases in dynamic interval occurred at the high current, where no decrease in the maximum rates was observed. Similarly, AA11 which showed significant decreases in response rate for the low current, failed to show any increases in the dynamic interval at that current while an increase in the dynamic interval was evident at the middle current, where no changes were observed in the maximum rates. In the case of AA28, the dynamic intervals were more variable at the low current, but this variability did not appear to be related to the variability in the maximum rates. For example, there is no difference in the maximum rate between session 1 and 9, while there is a significant increase in the dynamic interval for session 9, not apparent in session 1. Furthermore, although there are some increases in the dynamic interval at the high current, no changes are evident in the maximum rates.

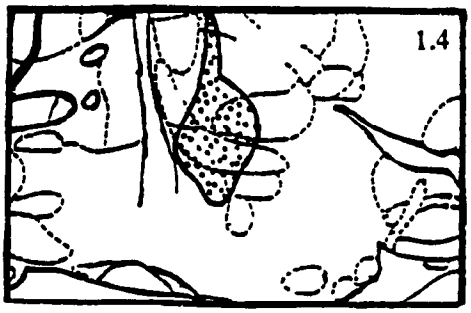
Lesion Location

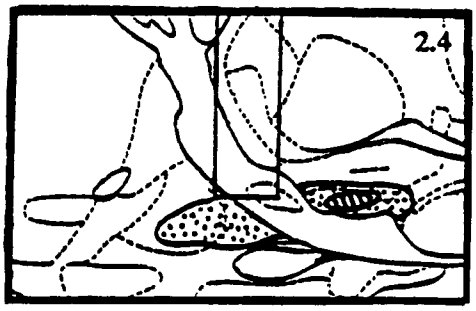
Histological data for subjects in this group are shown in Figs. 13-15. In all cases, NMDA lesions destroyed at least some of the LPOA, ALH and substantia innominata (SI). Two subjects, AA11 and AA30, had damage surrounding the internal capsule (IC). The subthalamic nucleus (STh) was destroyed in the case of AA11 while the reticular thalamic nucleus (Rt) and parts of the zona incerta (ZI), and the ventrolateral thalamic nucleus (VL) were damaged in the case

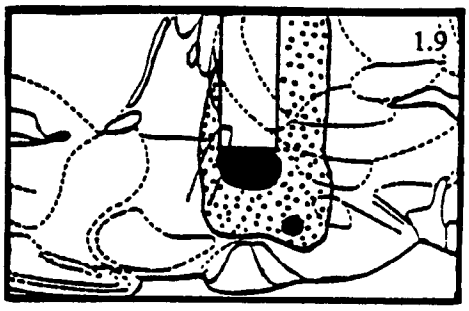
Figs. 13-15. Lesion and electrode tip locations for subjects with large increases in the required number (Group A). Reconstructions were made onto tracings of sagittal plates from the Paxinos and Watson atlas (1986). The alphanumeric at the bottom of each column identifies the subject. The distance of each plate from midline is given in the upper righthand corner. The reconstructions show the most representative sections from the plates corresponding to the first and the last substantial sign of the lesion, as well as the largest cross-section. The electrode locations are marked by filled circles. Cell loss, demyelination, and holes are represented by the stippled, hatched, and blackened areas, respectively.

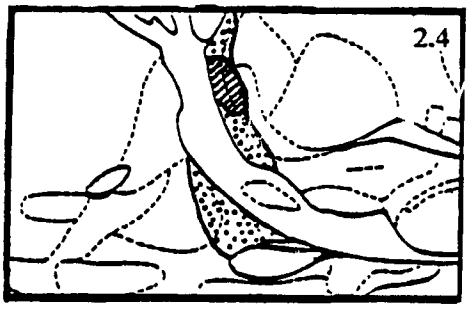






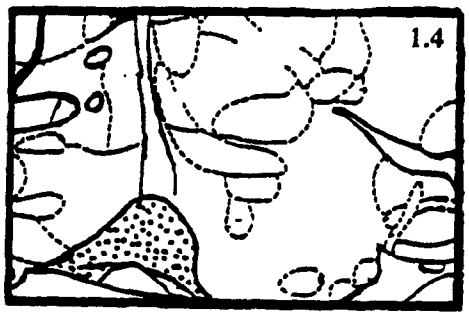


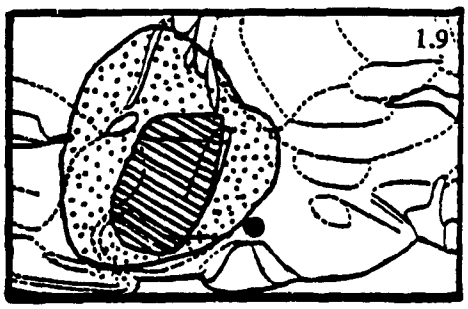




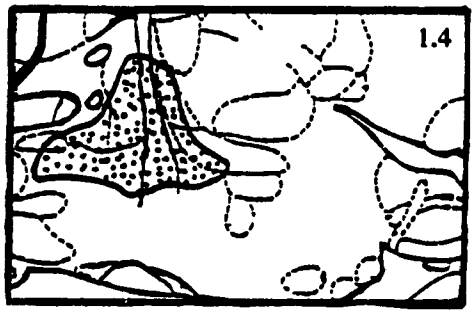
AA11

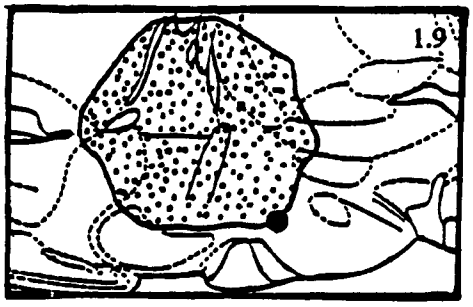
AA30





AA28 **AA37**





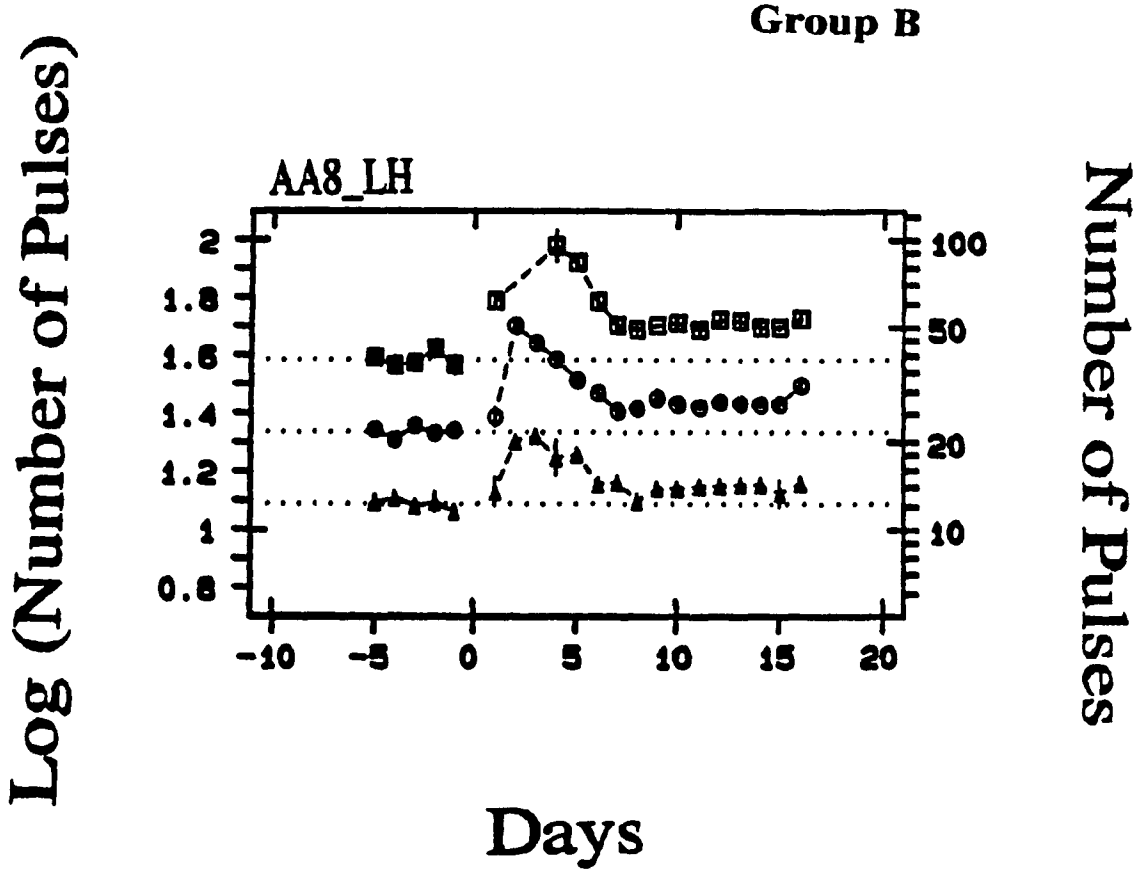
AA39

of AA30. A small area of demyelination around the Rt and the VL, well removed from the electrode tip, was observed for AA30. As well, a small hole lies beneath the tip of the cannula. The lesions in subjects AA28, AA37 and AA39 were centered around the LPOA and SI and extended to varying degrees to the the nucleus accumbens (Acb), the globus pallidus (GP), the ventral pallidum (VP), the bed nucleus of the stria terminalis (BST), the nucleus of the horizontal limb of the diagonal band (HDB), and the LH. Subject AA28 had the best defined lesion from amongst these three rats. In rat AA37, demyelination enveloped the entire LPOA and SI but remained away from the electrode tip. A small zone of demyelination restricted to a small portion of the SI occurred in AA28. Notably, the zone of demyelination was always smaller than the zone of cell loss, a finding similar to that of Stellar et al. (1991). No subject in Group A exhibited nonspecific damage (holes) or demyelination encroaching on the electrode tip.

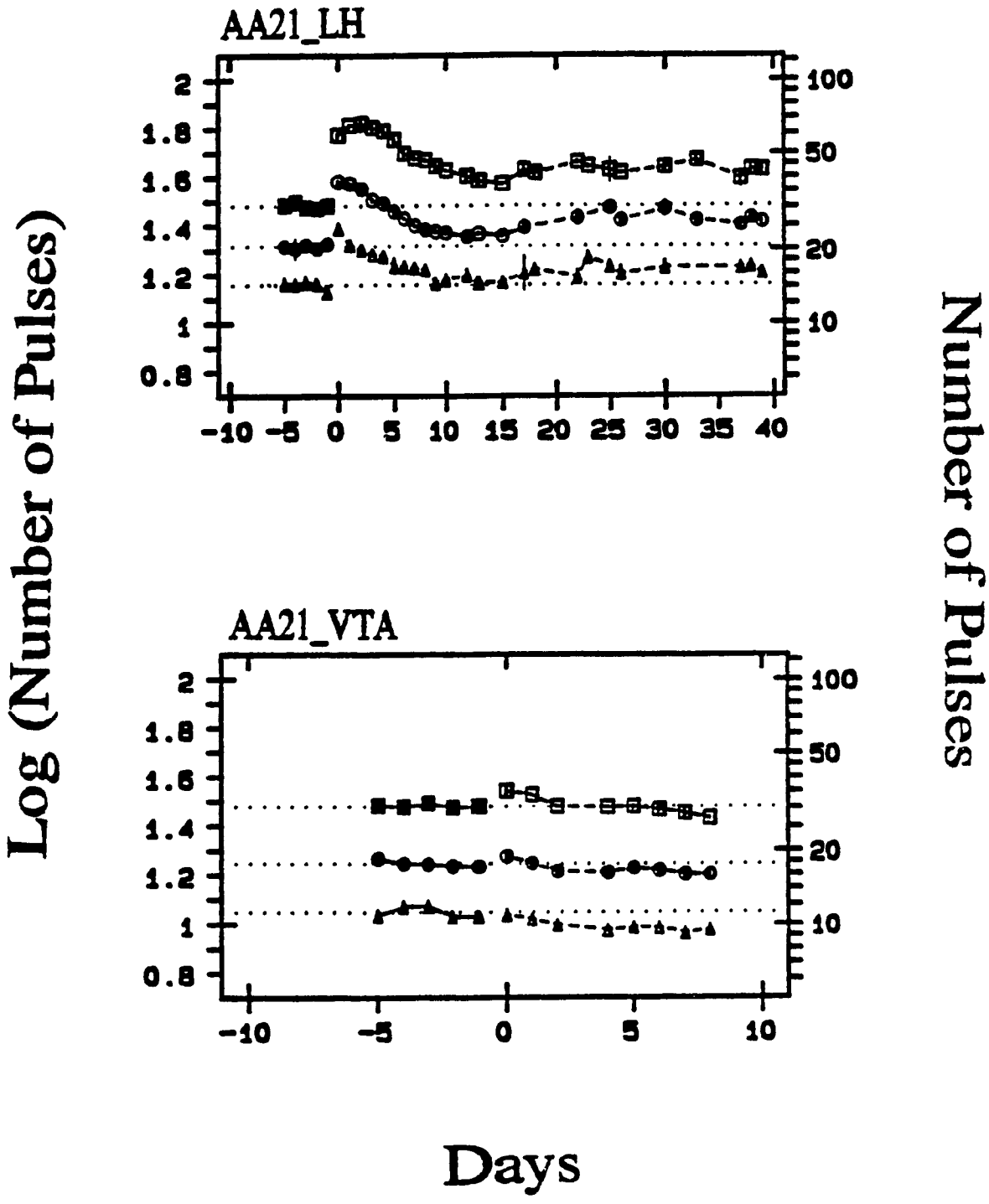
Group B: Substantial increases in the required number

This group consists of 7 rats (AA8, AA21, AA12, AA13, AA16, AA22, AA29). Self-stimulation was supported only at the LH site in subjects AA8, AA22, and AA29, with the rest of the subjects responding for stimulation of both the LH and VTA. The required number data for subjects in group B are shown in Figs. 16-21. In two cases, (AA8 and AA21, LH site)

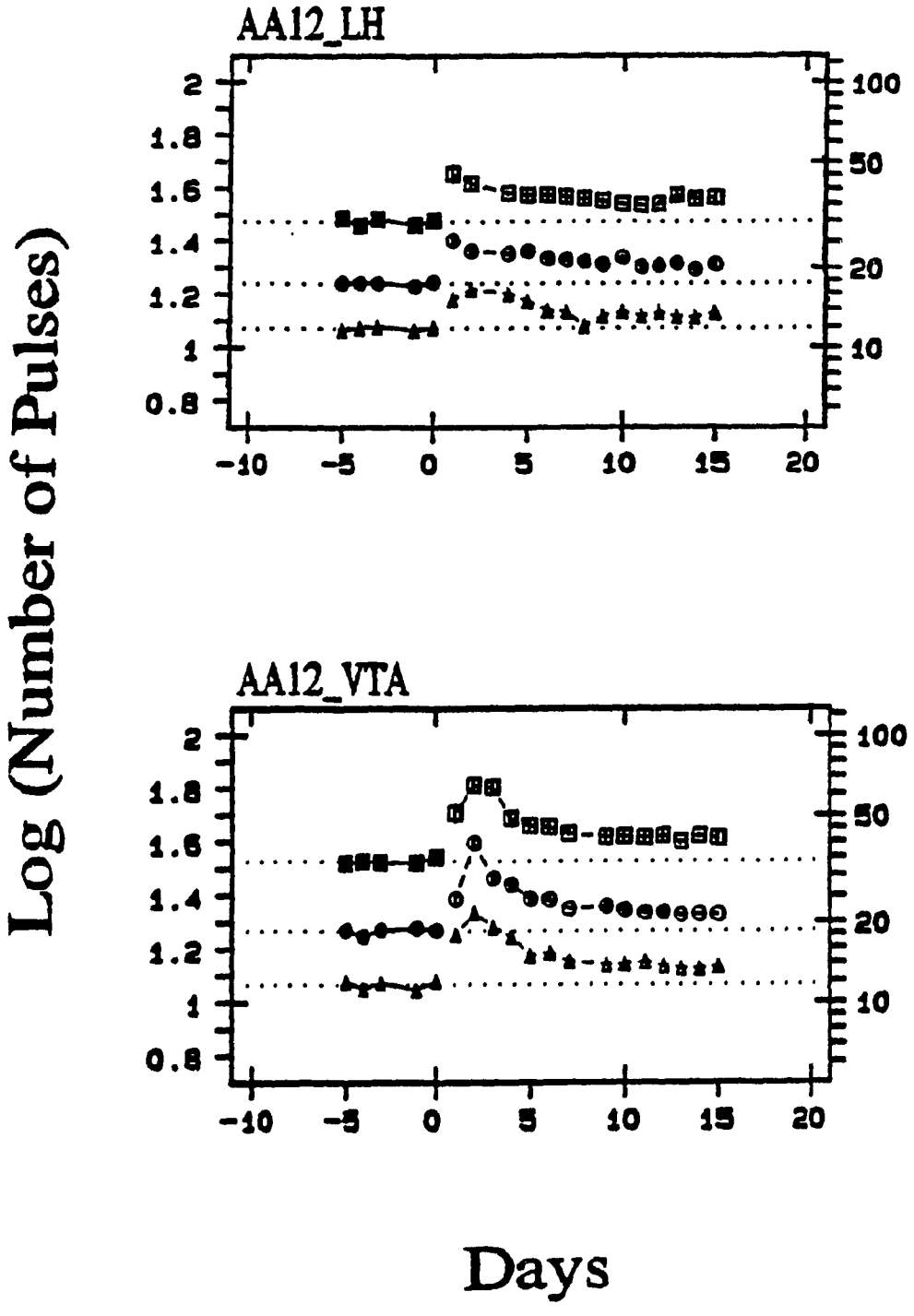
Fig. 16-21. Effects of lesion on self-stimulation in subjects AA8, AA21, AA12, AA13, AA16, AA22, and AA29 (Group B). The lesion in these subjects produced substantial increases in the required number. Each subject is identified in the top-left corner of the graph. Prelesion data are designated by negative values along the abscissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last prelesion test session. Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


Group B
AA8_LH
Log (Number of Pulses)
Number of Pulses
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10
15
20
Days

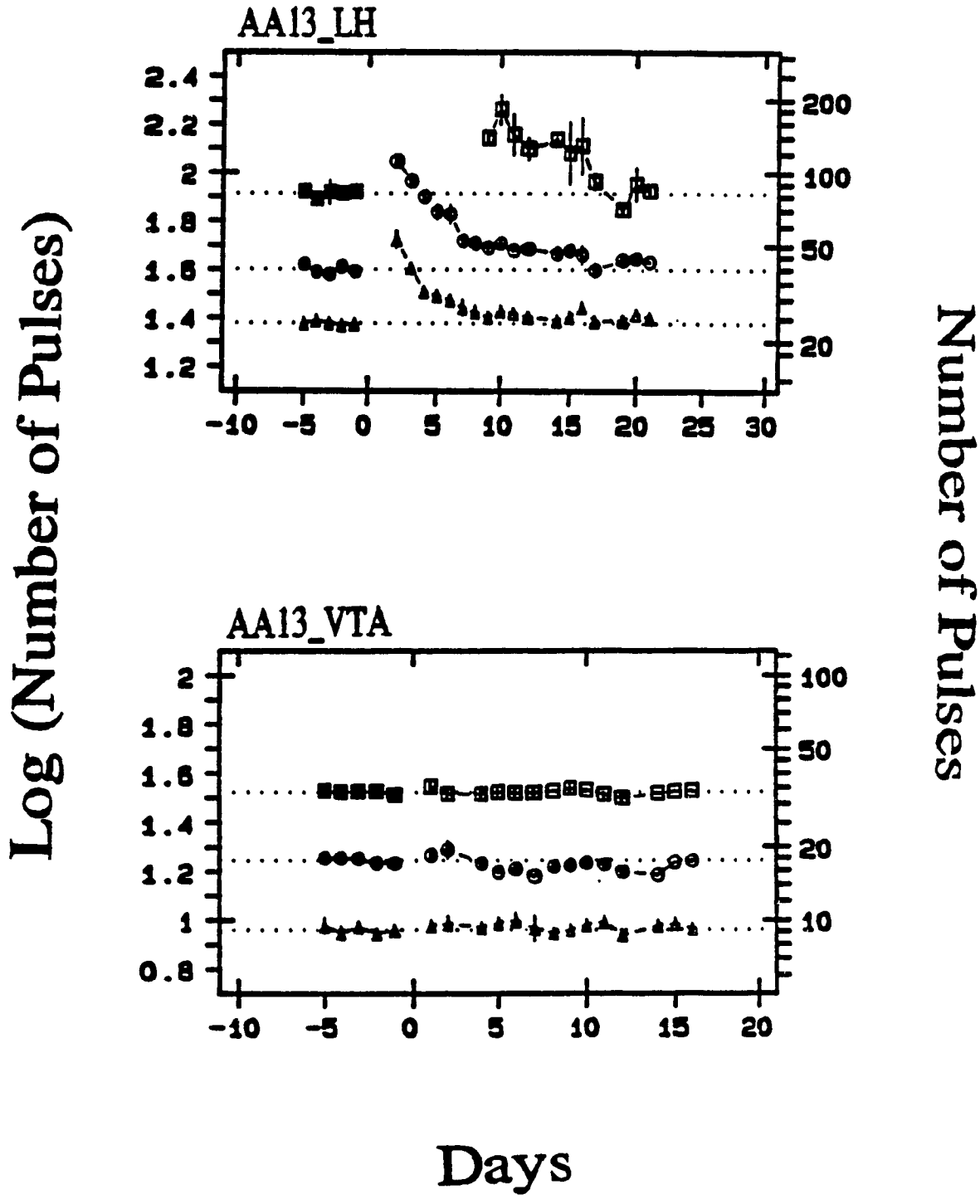
Group B


AA21_LH
AA21_VTA
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10 -5 0 5 10 15 20 25 30 35 40
-10 -5 0 5 10

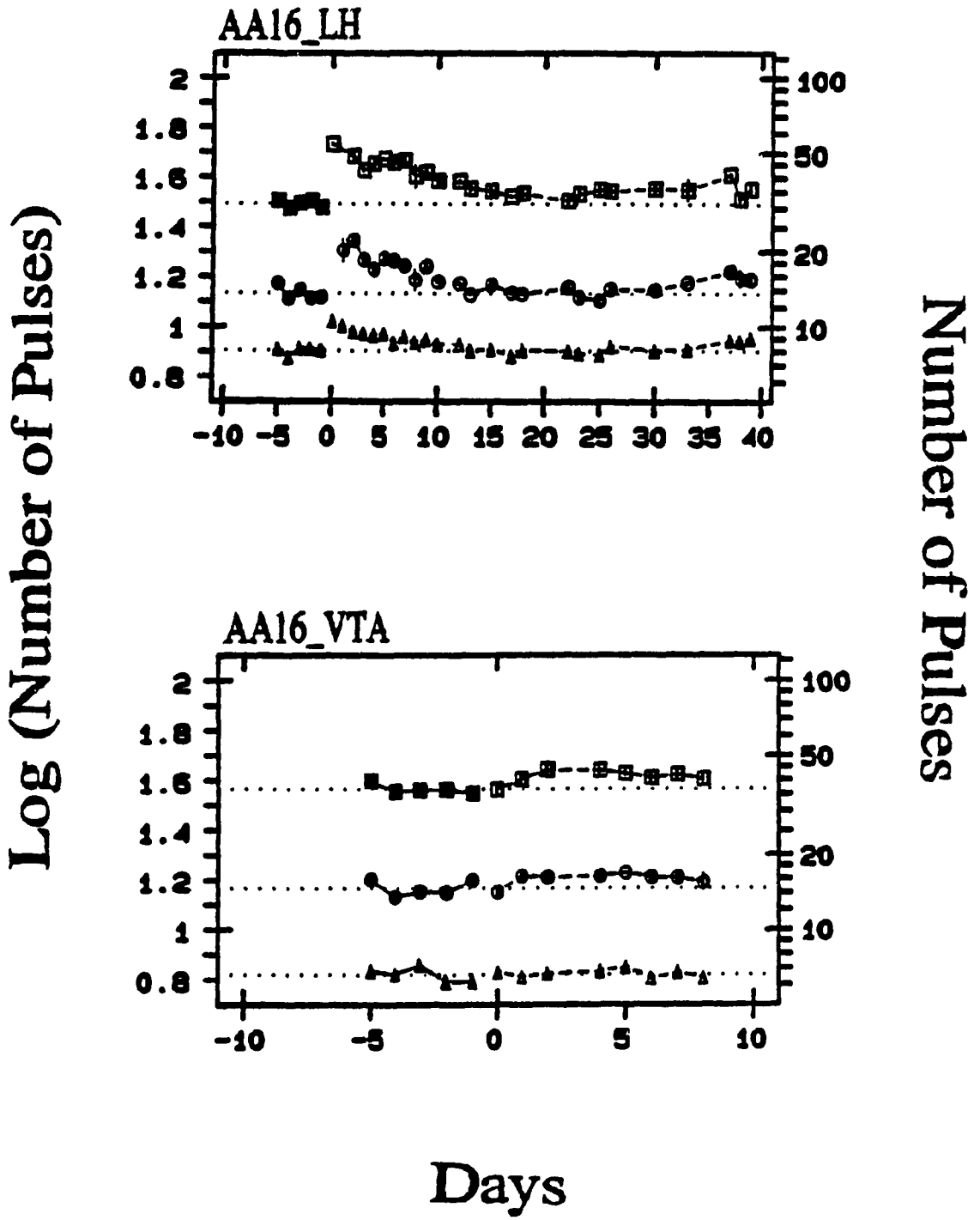
Group B


AA12_LH
AA12_VTA
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10
15
20

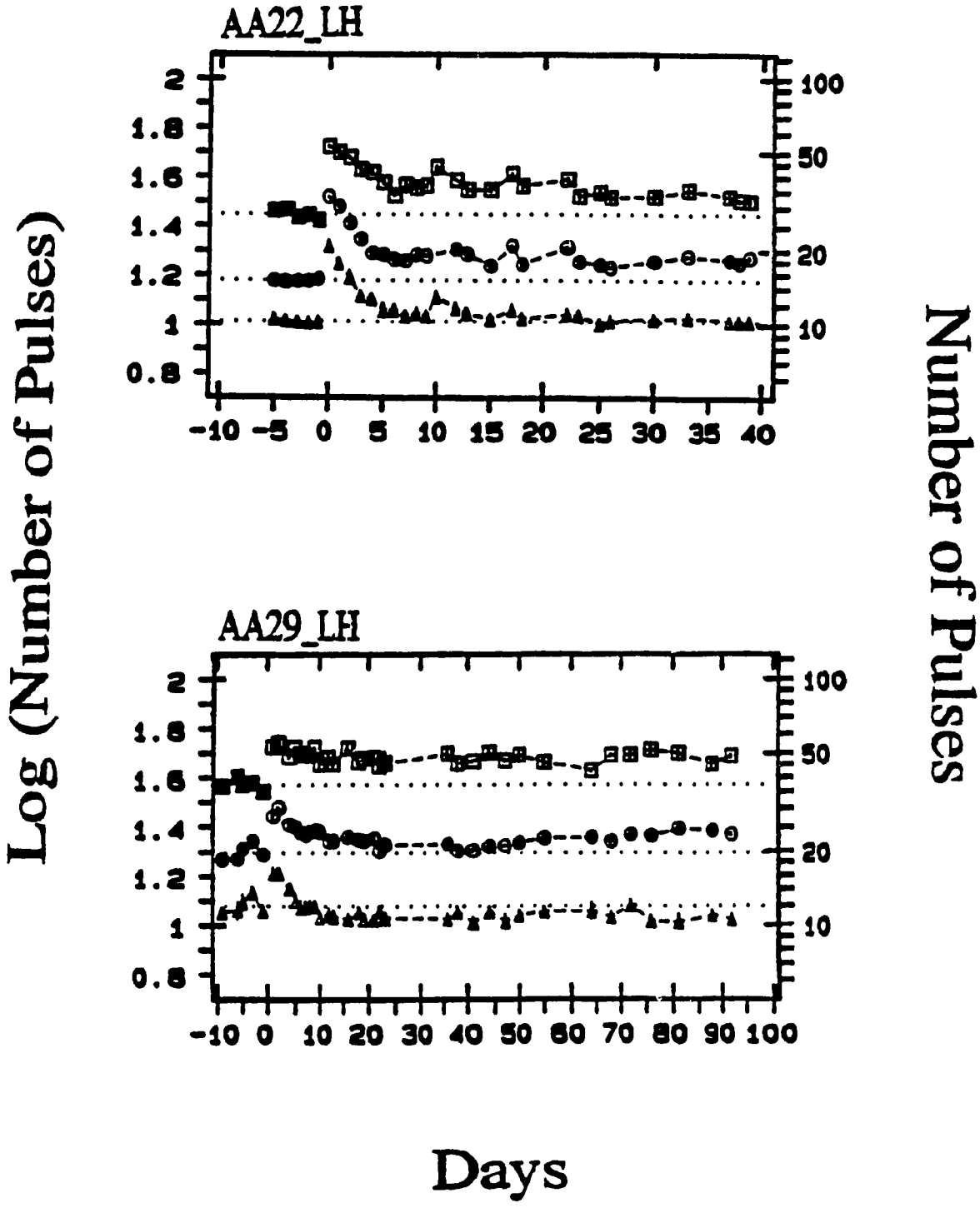
Group B


AA13_LH
2.4
2.2
2
1.8
1.6
1.4
1.2
200
100
50
20
-10
-5
0
5
10
15
20
25
30
AA13_VTA
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10
15
20
Log (Number of Pulses)
Number of Pulses
Days

Group B


AA16_LH
AA16_VTA
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10 -5 0 5 10 15 20 25 30 35 40
-10 -5 0 5 10

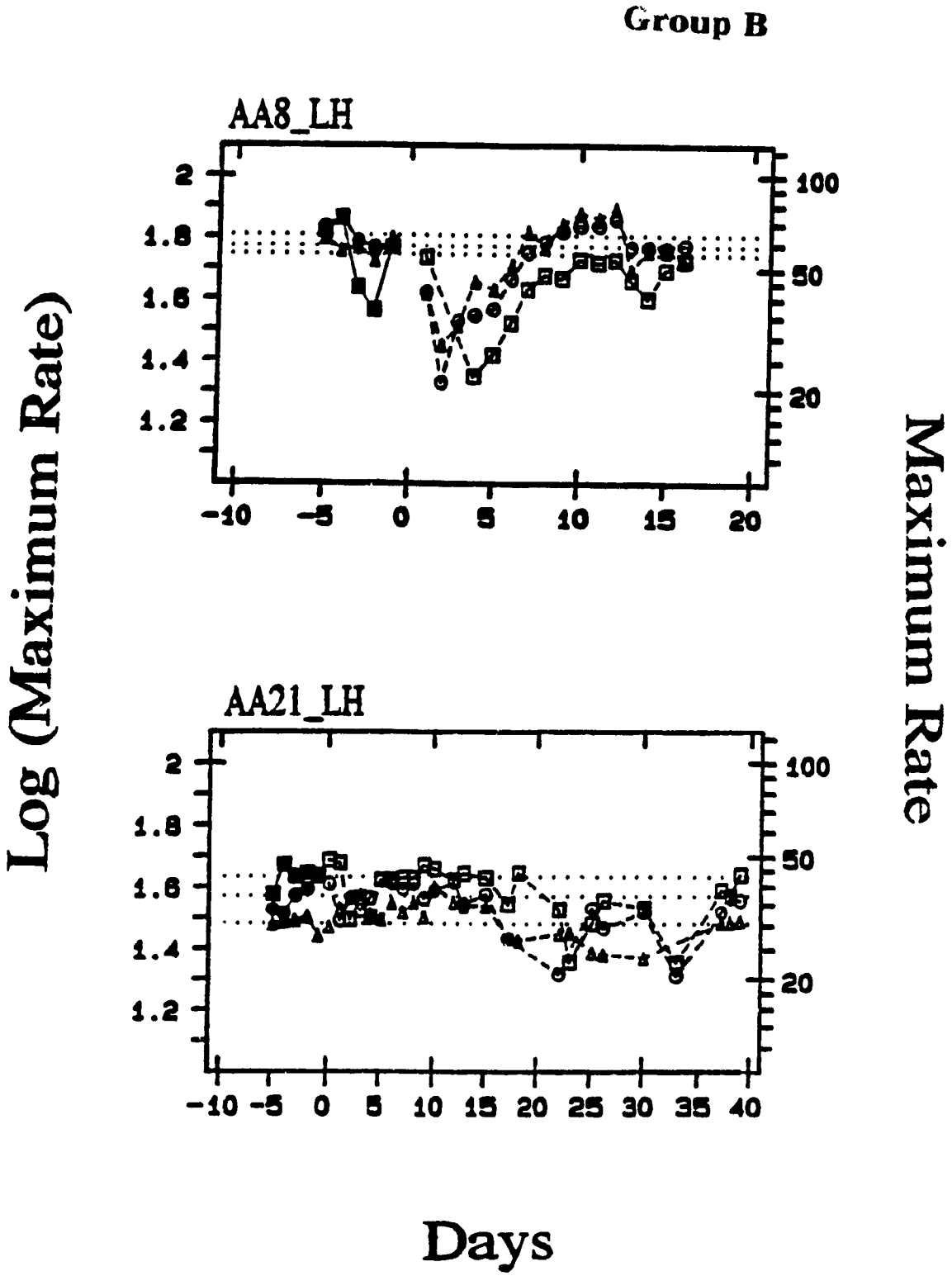
Group B

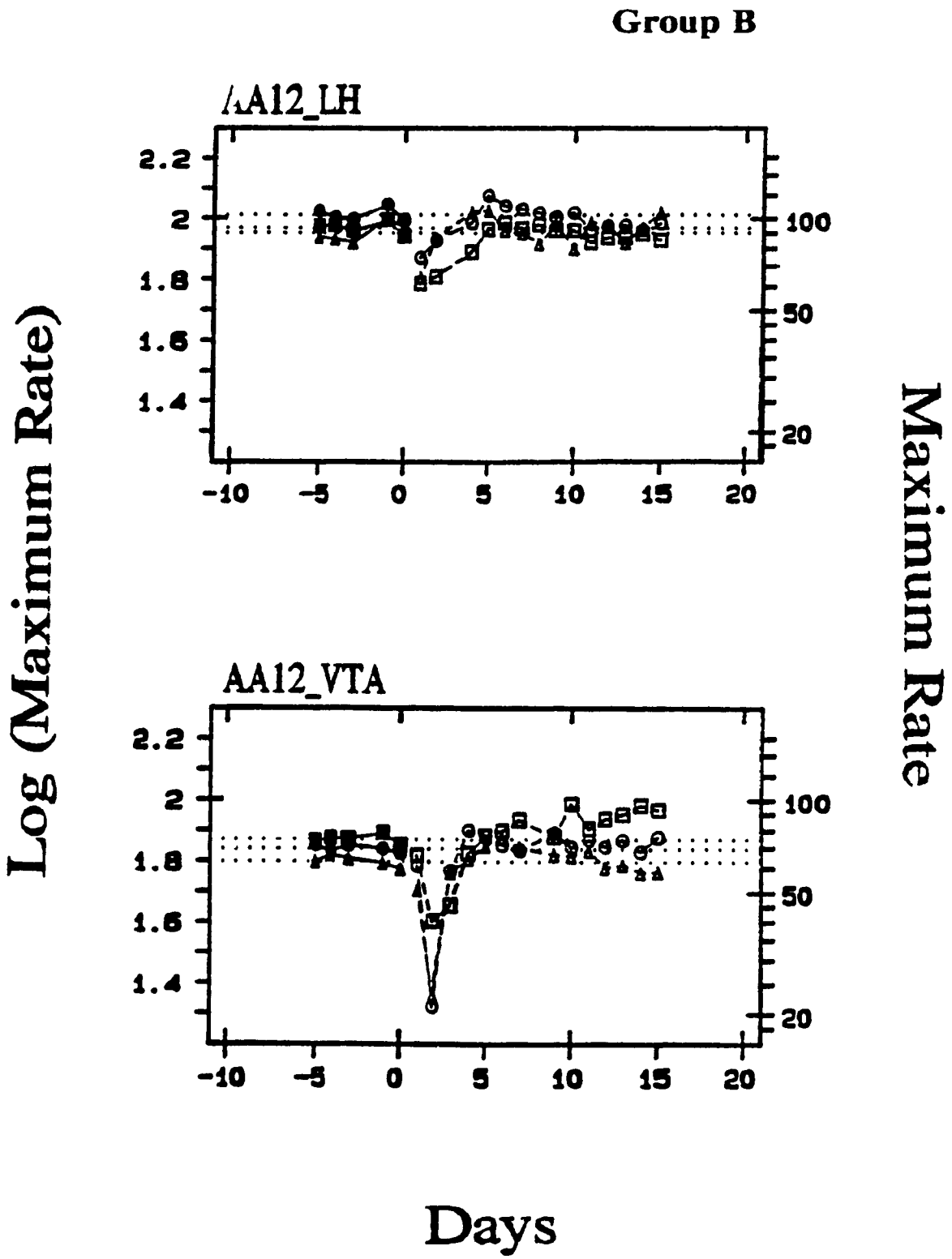

AA22_LH
AA29_LH
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10 -5 0 5 10 15 20 25 30 35 40
-10 0 10 20 30 40 50 60 70 80 90 100

lesions produced a large immediate postlesion increase at all currents (0.30-0.40 $\log_{10}$ units), that subsequently stabilized at the lower current at approximately 0.10 $\log_{10}$ units above baseline. No changes in the required number were observed in the case of AA21 at the VTA site. AA12, AA22 and AA29 showed increases in the required number immediately following the lesion, ranging from 0.15 $\log_{10}$ units to more than 0.3 $\log_{10}$ units, that subsequently recovered to below 0.1 $\log_{10}$ units. It is noteworthy that substantial increases in threshold were observed immediately following the lesion, for stimulation delivered through both the LH and VTA electrodes in the case of subject AA12. The effect was largest at the VTA site. Subjects AA13 and AA16 showed no changes for stimulation at the VTA site whereas pronounced transient increases in required number were observed at the LH site. AA13 failed to self-stimulate for more than a week following the lesion at the lower current, whereas an immediate postlesion increase in threshold of approximately 0.4 log10 units was observed at the other currents.

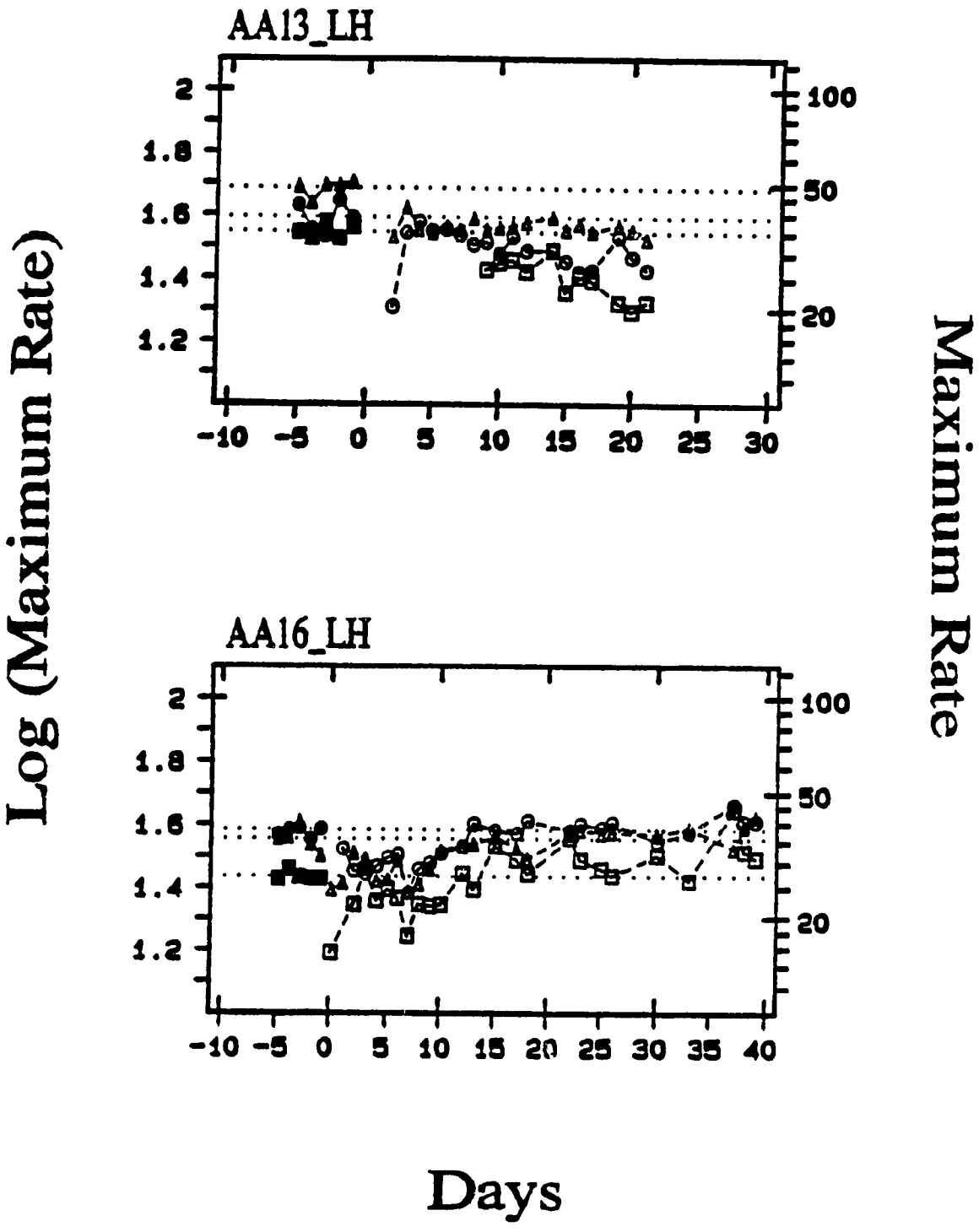
The maximum response rates for subjects in group B are shown in Figs. 22-25. Analysis of maximum rates was performed only for self-stimulation sites where transient increases in the required number threshold were observed. In most subjects, the transient increase in the required number was accompanied by a corresponding transient decrease in

Figs. 22-25. Maximum rates for subjects AA8, AA21, AA12, AA13, AA16, AA22, and AA29 (Group B). The lesion in these subjects produced substantial increases in the required number. Each subject is identified in the top-left corner of the graph. Prelesion data are designated by negative values along the abscissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last prelesion test session. Postlesion data are represented by open symbols. Error bars are omitted.

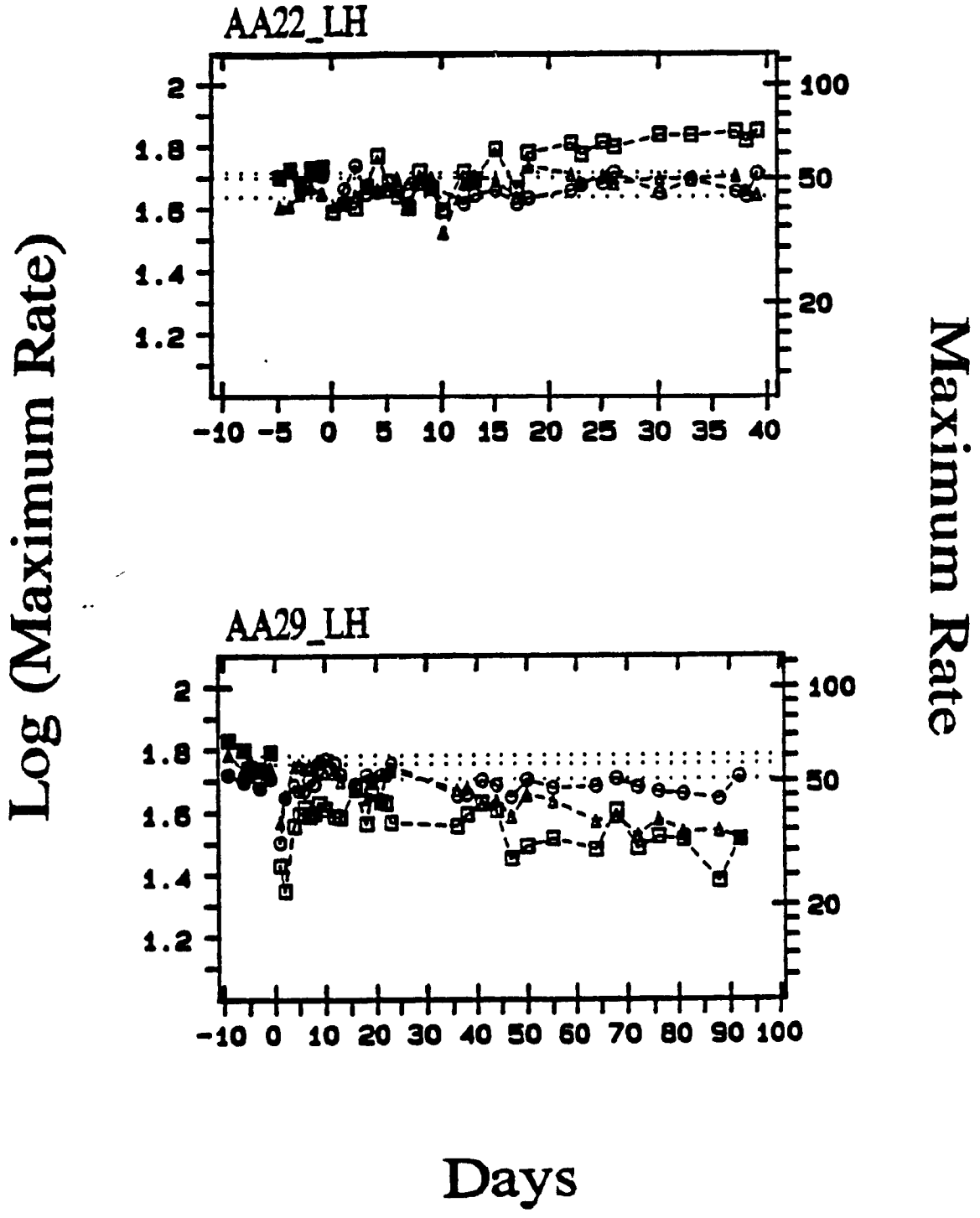

Group B
AA8_LH
AA21_LH
Log (Maximum Rate)
Maximum Rate
Days
2
1.8
1.6
1.4
1.2
100
50
20
-10 -5 0 5 10 15 20
-10 -5 0 5 10 15 20 25 30 35 40


Group B
AA12_LH
2.2
2
1.8
1.6
1.4
100
50
20
-10
-5
0
5
10
15
20
AA12_VTA
2.2
2
1.8
1.6
1.4
100
50
20
-10
-5
0
5
10
15
20
Log (Maximum Rate)
Maximum Rate
Days

Group B


AA13_LH
AA16_LH
Log (Maximum Rate)
Maximum Rate
Days
2
1.8
1.6
1.4
1.2
100
50
20
-10 -5 0 5 10 15 20 25 30
-10 -5 0 5 10 15 20 25 30 35 40

Group B


AA22_LH
AA29_LH
Log (Maximum Rate)
Maximum Rate
Days
2
1.8
1.6
1.4
1.2
100
50
20
-10 -5 0 5 10 15 20 25 30 35 40
-10 0 10 20 30 40 50 60 70 80 90 100

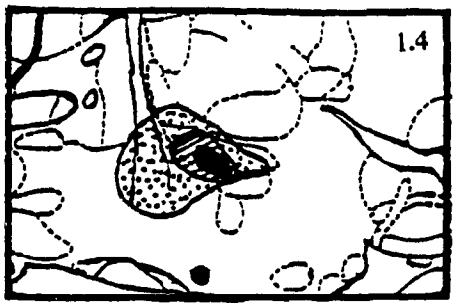
maximum response rates. This is quite striking in the case of subjects AA8, and AA12 at the VTA site, but is also observed for AA12 at the LH site and AA16. Quite the opposite phenomenon occurs in the case of AA13, where the transient change in the required number was not accompanied by decreases in the maximum rates. However, at the point at which stabilization in the required number threshold occurs, decreases in the maximum rates start to be evident. A significant decrease in maximum rate at the low current, and at the high current after session 20, was observed for AA29. Subject AA21 had no depression in the maximum response rate immediately following the lesion when a transient increase in the required number was seen. Interestingly, at the point where the increase in the required number stabilized, the maximum rates begin to significantly decrease for this subject. No decreases in the maximum response rate were observed in the case of AA22. In some subjects, there appears to be a relationship between the transient increases in the required number and decreases in maximum rates. When stabilization in the required number threshold occurs, even at some level above baseline, maximum rates also seem to return to more normal values.

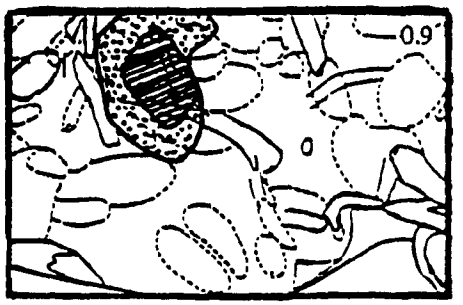
Lesion Location

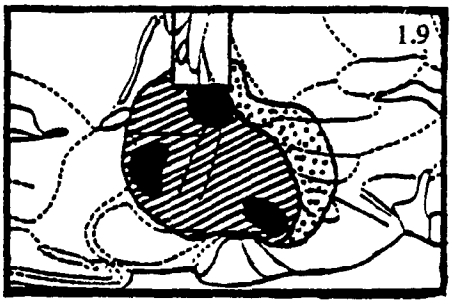
The lesions in group B were similar in location to those observed in the first group of subjects. Histological

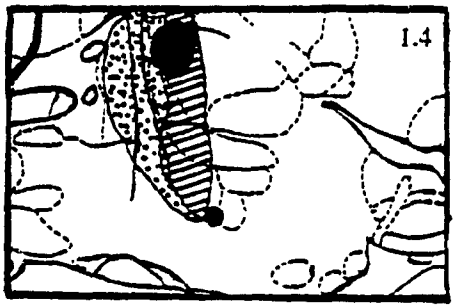
reconstructions for group B are shown in Figs. 26-29. The lesions in subjects AA12, AA13, AA16, AA21, and AA22 destroyed tissue surrounding the IC such as parts of the SI, VP, GP, Rt, and ZI. Often, cell death extended more medially and was centered around the area between the BST and the Rt, above the stria medullaris (sm). It is hard to give an accurate description of those lesions because the lesion borders are confounded with the IC, a fibre tract populated by a large amount of glial cells. The zone of cell loss appears to be quite restricted, particularly for AA12, perhaps as a result of excitotoxin diffusion into the IC. Consequently, damage is also quite removed from the stimulating electrode, except in the case of AA21 and AA22. There appears to be a hole at the tip of the cannula in the case of AA12 and AA21 and a hole within the borders of the IC is evident in AA22. Furthermore, varying degrees of demyelination are evident in subjects AA13, AA16, AA21 and AA22. In the case of AA21, demyelination encroaches on the electrode tip. The rest of the subjects in this group, AA8 and AA29, had more ventrally located damage centred primarily in the SI, LPOA and ALH with damage occasionally extending to more dorsal structures. Nonspecific damage is striking in the case of AA8 where several holes are encompassed within the general area of cell loss and demyelination.

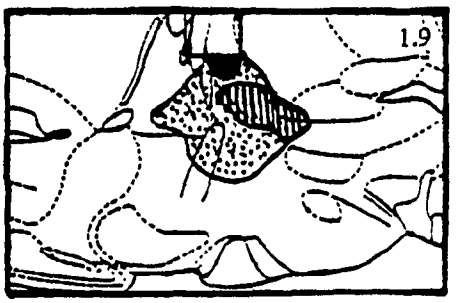
Figs. 26-29. Lesion and electrode tip locations for subjects with substantial increases in the required number (Group B). Reconstructions were made onto tracings of sagittal plates from the Paxinos and Watson atlas (1986). The alphanumeric at the bottom of each column identifies the subject. The distance of each plate from midline is given in the upper right-hand corner. The reconstructions show the most representative sections from the plates corresponding to the first and the last substantial sign of the lesion, as well as the largest cross-section. The electrode locations are marked by filled circles. Cell loss, demyelination, and holes are represented by the stippled, hatched, and blackened areas, respectively.



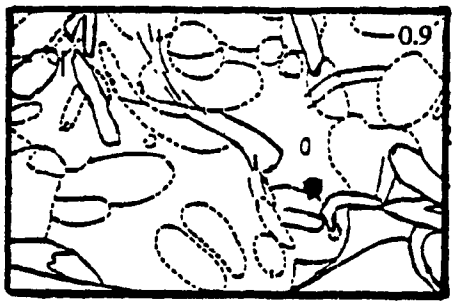


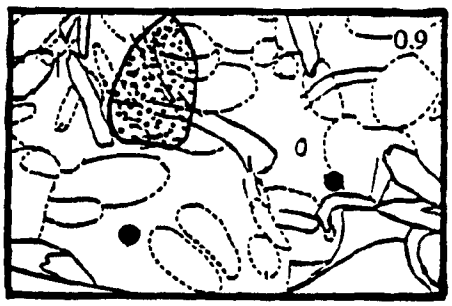


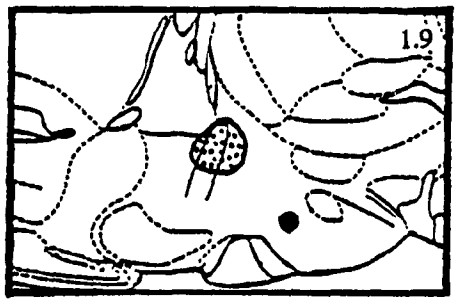


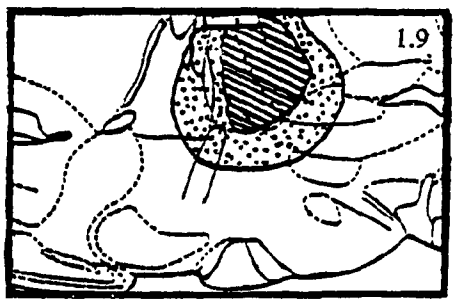


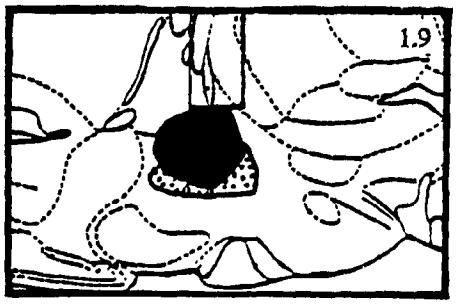
AA8 **AA21**

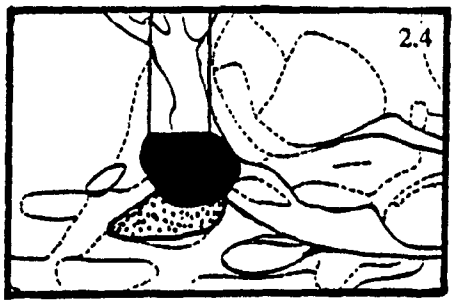




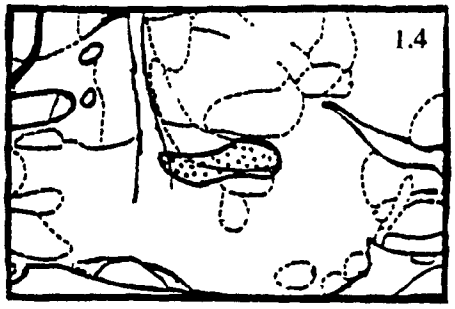


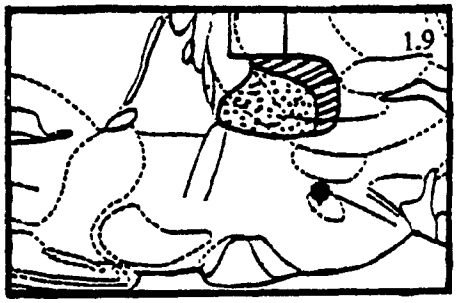


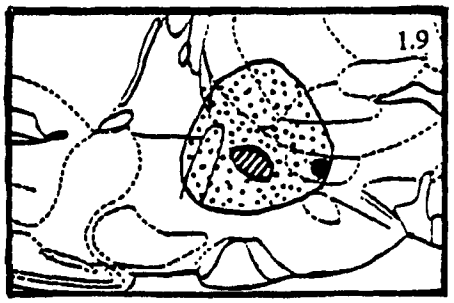


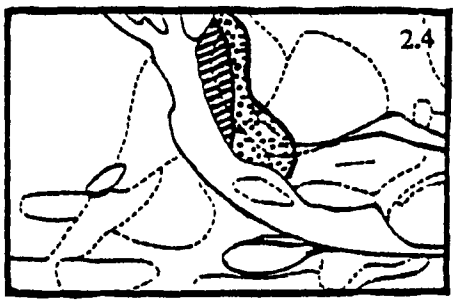


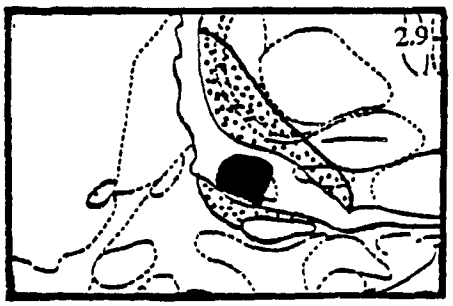
AA12 **AA13**



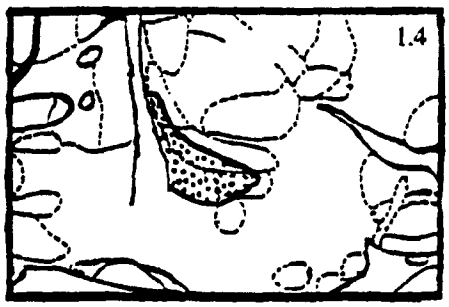


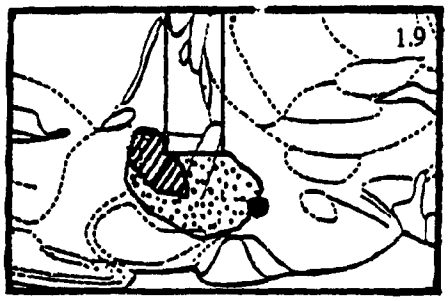






AA16 **AA22**





AA29

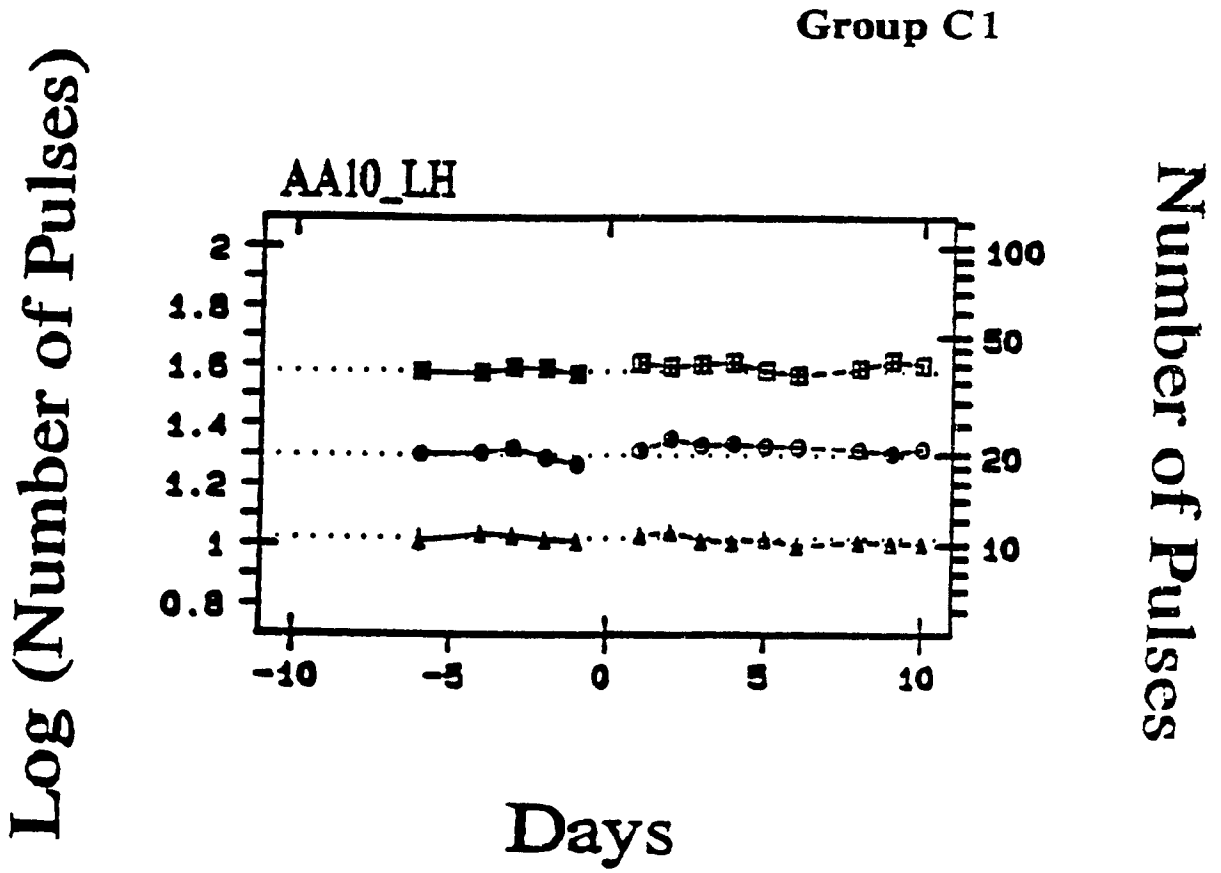
Group C: No increases in the required number

This group is composed of a sub-group of subjects that were lesioned with NMDA and a sub-group that received vehicle control injections. Results for the NMDA-treated animals (Group C1) are shown in Figs. 30-32 whereas results for the control subjects (Group C2) are shown in Figs. 33-34. Of the NMDA-treated animals, two, AA24 and AA27, self-stimulated at both the LH and VTA sites, while AA10 self-stimulated only at the LH. From the control subjects, AA35 self-stimulated at the LH and VTA while AA32 and AA33 self-stimulated only at the LH site. An absolute lack of effect characterizes the results from all these subjects.

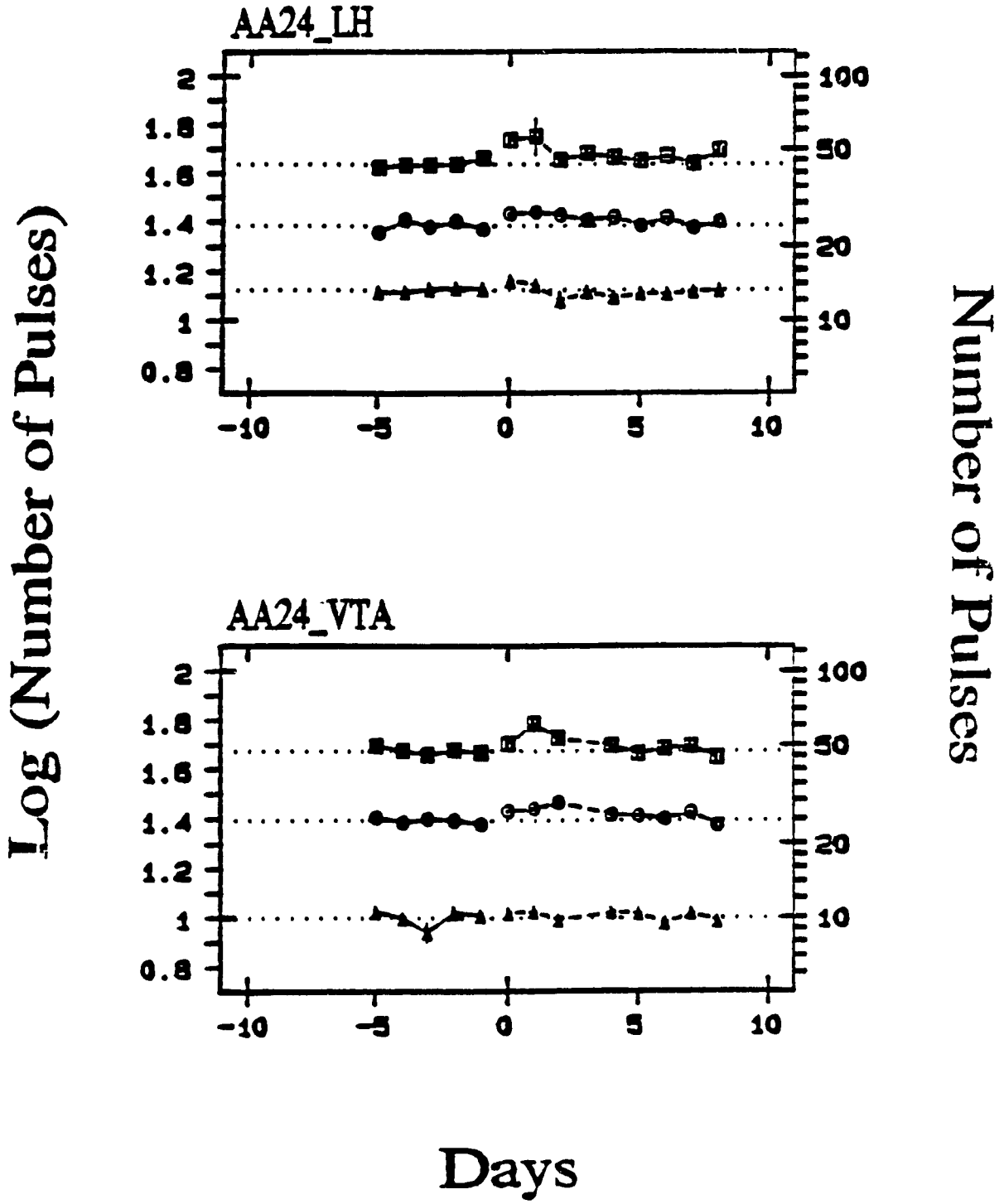
Lesion Location

Histological reconstructions for subjects in Group C1 that showed no increase in the required number are shown in Fig. 35. No damage was observed in the case of AA10, therefore no histology is included. Surprisingly, in the other two subjects that received NMDA injections, the regions damaged overlapped with areas destroyed in the first two groups of rats. More specifically, the damage for AA27 subsumed the SI, the GP, a part of the VP, the LPOA and, the BST. The other rat, AA24, had damage located dorsally to the MFB above the IC and SM. The control rats, AA32, AA33 and AA35, showed no evidence of neuronal damage or demyelination.

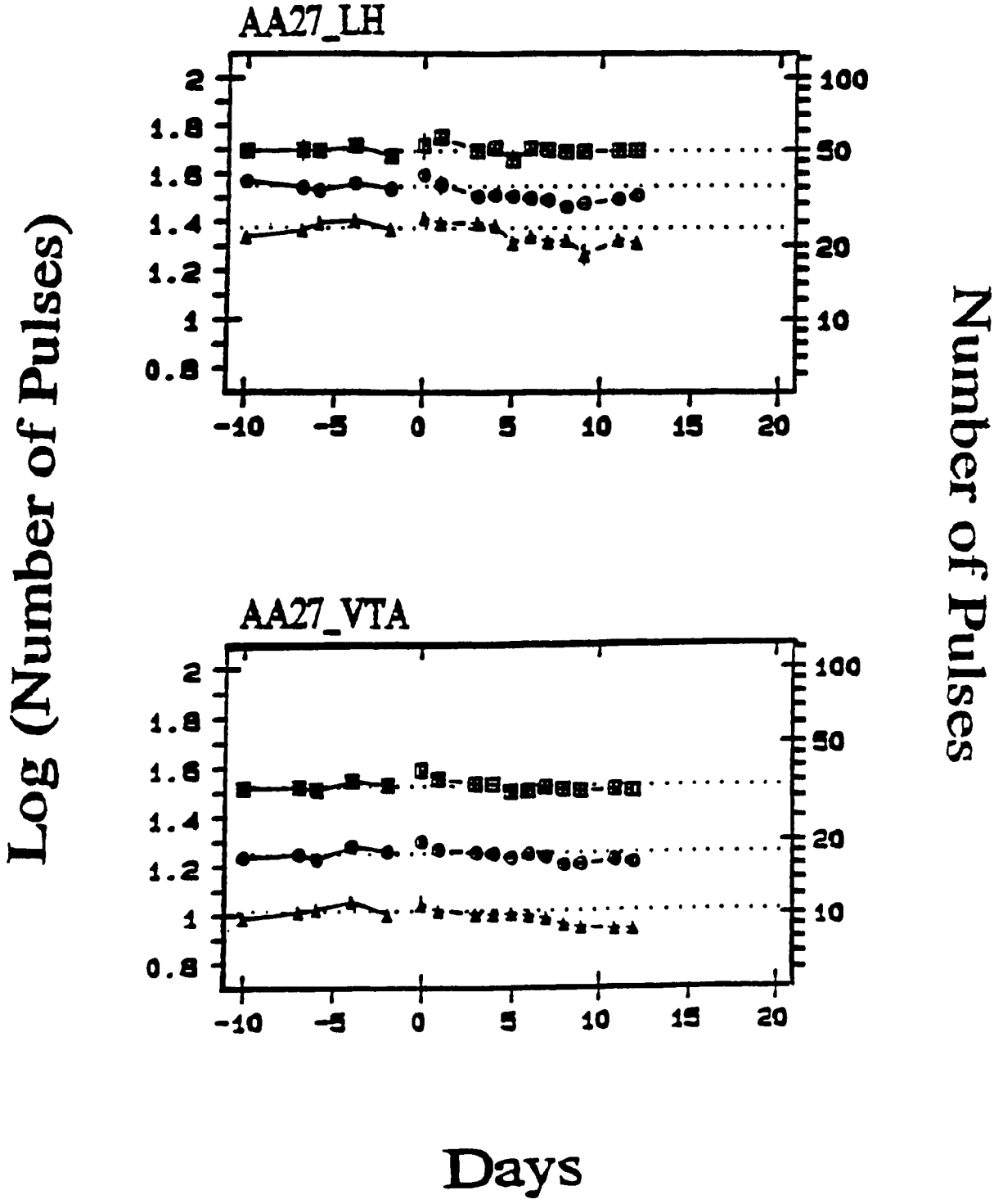
Figs. 30-32. Effects of lesion on self-stimulation in subjects AA10, AA24, AA27 (Group C1). The lesion in these subjects produced no increases in the required number. Each subject is identified in the top-left corner of the graph. Prelesion data are designated by negative values along the abscissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last pretlesion test session. Postlesion data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


Group C1
AA10_LH
Log (Number of Pulses)
Number of Pulses
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10
Days

Group C1

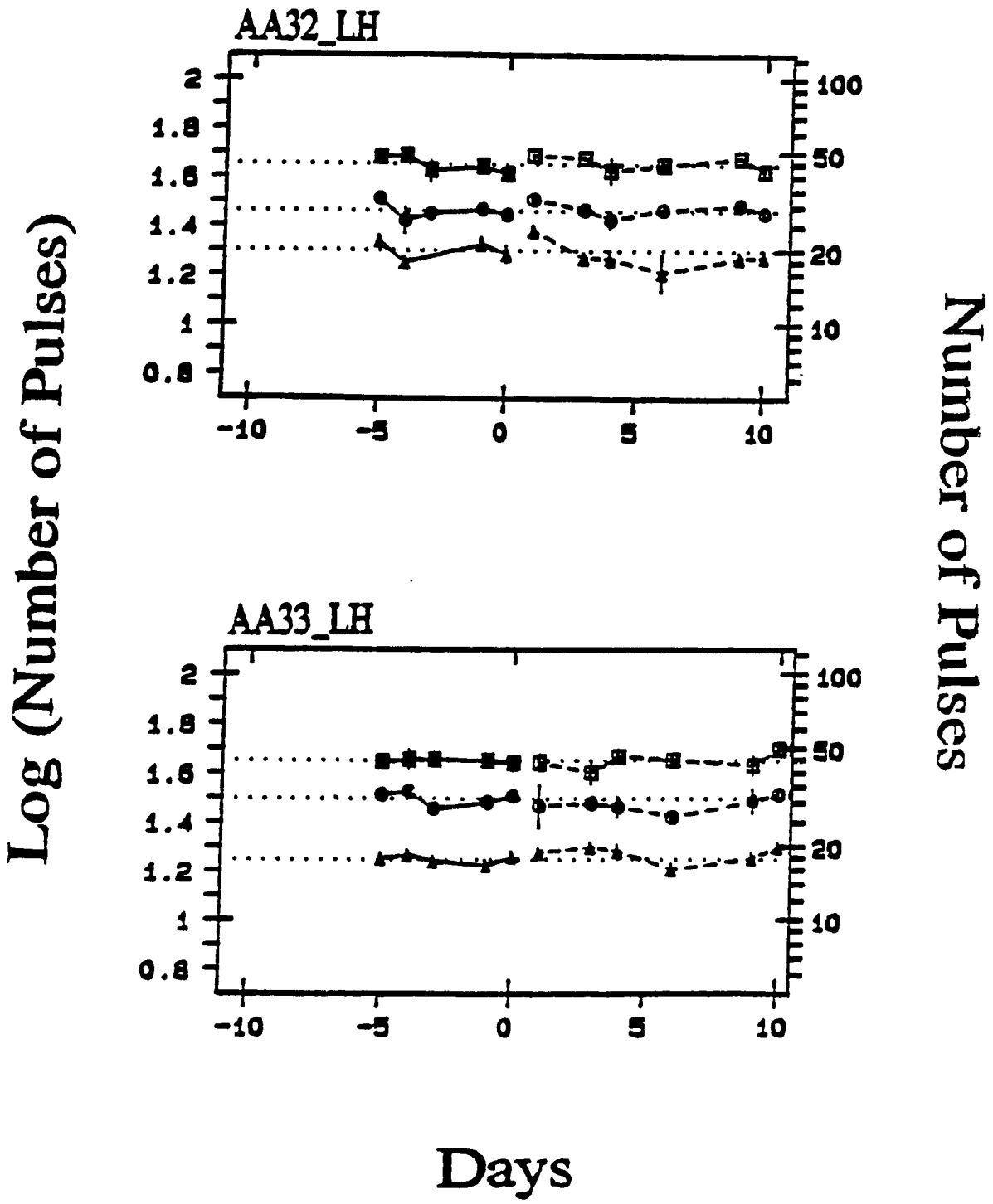

AA24_LH
AA24_VTA
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10

Group C1


AA27_LH
AA27_VTA
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10
15
20

Figs. 33-34. Effects of control injections on self-stimulation in subjects AA32, AA33, AA35 (Group C2). No increases in the required number were observed. Each subject is identified in the top-left corner of the graph. Preinjection data are designated by negative values along the abscissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Injections were made after the last preinjection test session. Postinjection data are represented by open symbols. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

Group C 2


AA32_LH
AA33_LH
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10

Group C 2

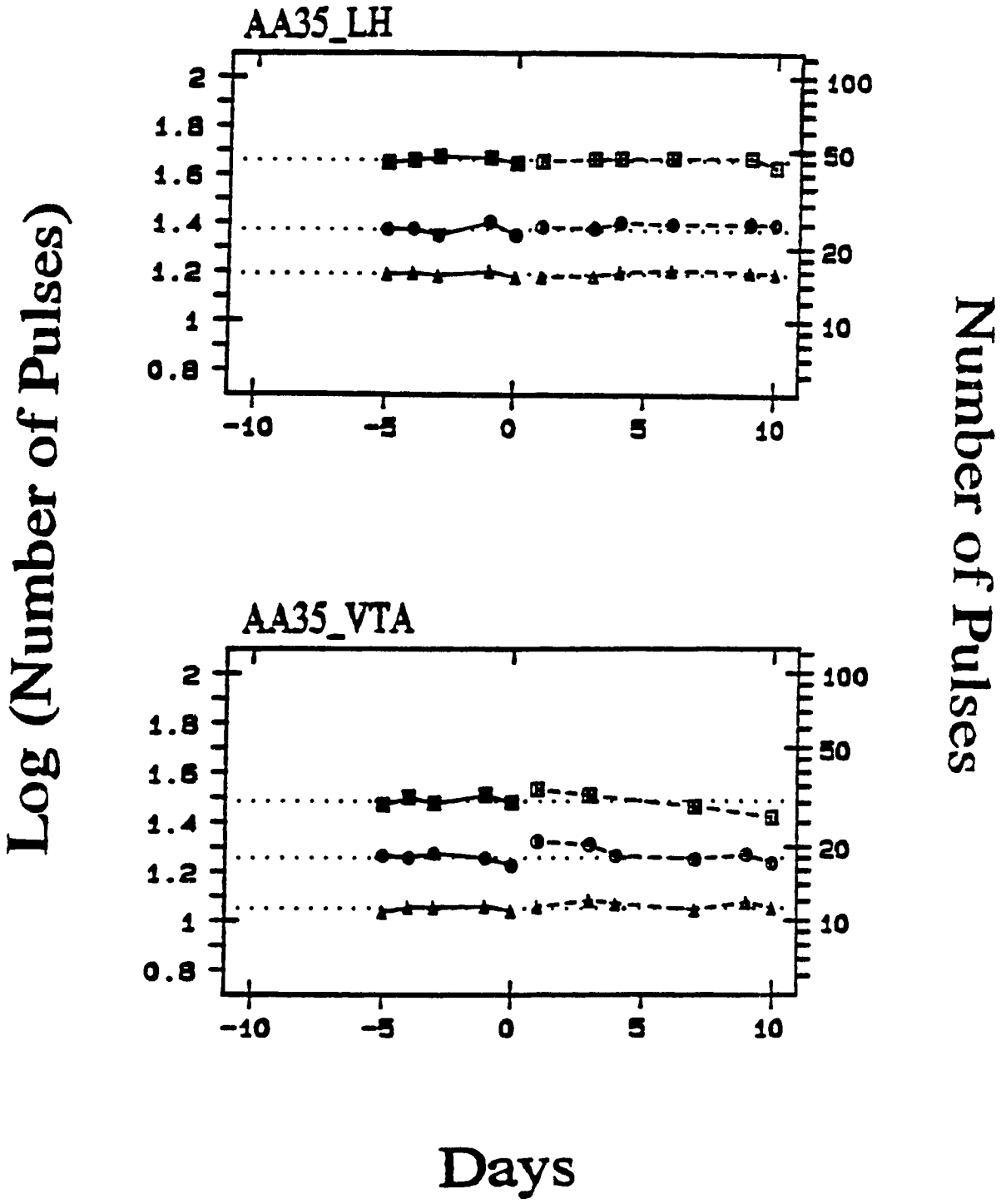
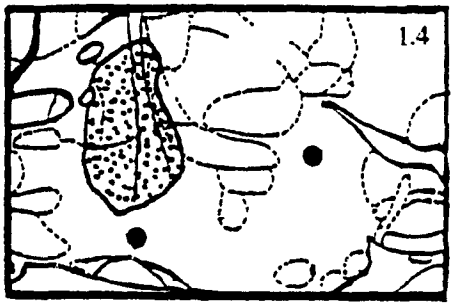
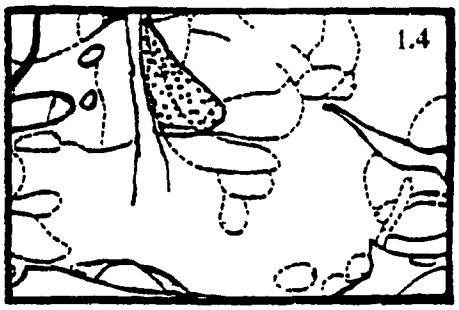
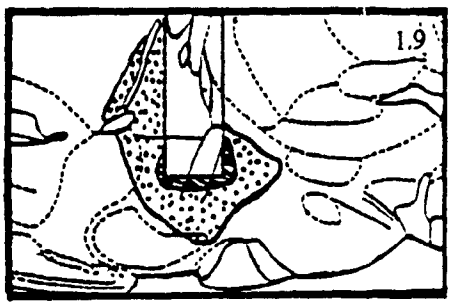
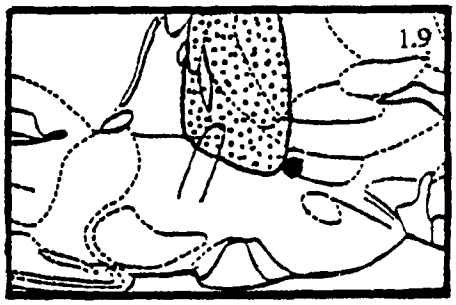

AA35_LH
AA35_VTA
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
0.8
100
50
20
10
-10
-5
0
5
10

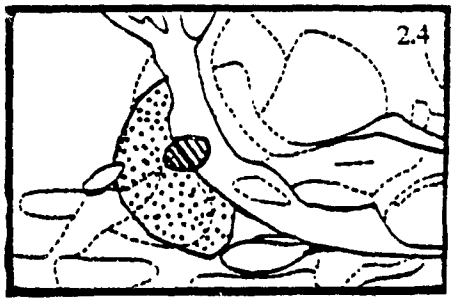
Fig. 35. Lesion and electrode tip locations for subjects with no increases in the required number (Group C1). Reconstructions were made onto tracings of sagittal plates from the Paxinos and Watson atlas (1986). The alphanumeric at the bottom of each column identifies the subject. The distance of each plate from midline is given in the upper righthand corner. The reconstructions show the most representative sections from the plates corresponding to the first and the last substantial sign of the lesion, as well as the largest cross-section. The electrode locations are marked by filled circles. Cell loss, demyelination, and holes are represented by the stippled, hatched, and blackened areas, respectively.

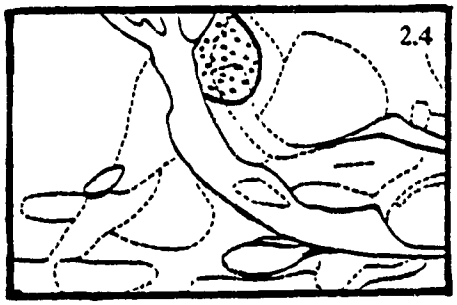












AA24 **AA27**

Discussion

The recent study by Murray and Shizgal (1991) has demonstrated that anterolateral electrolytic lesions of the MFB increase the required frequency for self-stimulation at LH and VTA sites. One of the weaknesses of electrolytic lesions is that one cannot be certain of whether cell bodies or axons of passage are responsible for the attenuation of self-stimulation. Considering this limitation and the amount of evidence accumulated over the years in favour of the use of excitotoxins as a means to create cell-body specific damage, an extension of the findings of the aforementioned study was undertaken using NMDA as the lesioning tool. The results obtained in the present study support and broaden the earlier work, showing that NMDA lesions of the basal forebrain produce long-lasting increases in the threshold for self-stimulation of the LH. Lesions in 5 of the 15 NMDA-treated subjects produced large increases ranging from 0.2 $\log_{10}$ units to more than 0.4 $\log_{10}$ units, equivalent, under the theoretical constraints of the counter model, to the destruction of 37-60% of the reward substrate. Seven other rats showed substantial increases of a transient nature.

Significance of Large Effects

The interpretation of required number as an index of reward efficacy depends on the stability of the asymptotic

response rate and the dynamic interval as previously discussed. The required number shifts for rats belonging in Group A are sizable enough to undermine any serious doubts concerning their interpretation. The highest shifts produced by manipulations of task difficulty range only between 0.1 to 0.2 $\log_{10}$ units (Miliaressis et al., 1986). Nevertheless, the contribution of performance variables on the postlesion rate-frequency functions cannot be completely ruled out without examining changes in the various parameters of the rate-frequency curves such as maximum rates and dynamic interval. Maximum response rates did not change significantly for one rat (AA30) while they were reduced mainly at the low current for the other 4 rats. There was no marked depression in response rates at the higher currents, except for subject AA39. Notably, in some subjects (e.g. AA11 and AA37), increases in the required number at the middle current were not accompanied by corresponding decreases in maximum rates. Dynamic interval analysis for these 4 rats revealed that there was no systematic change in the dynamic interval of the rate-frequency curves. For subject AA28, dynamic intervals appeared to be more variable between sessions, with some significant increases in certain sessions. Since either the maximum response rates or the dynamic intervals remained within baseline values after the lesion, it can be concluded that most likely, changes in the required number reflect real changes in reward and not changes in performance capacity.

Significance of Transient Effects

Several of the postlesion effects in this study were of a transient nature. An interesting finding of this study concerns the direct link between reward effectiveness and performance, observed mainly within the group of rats with substantial increases in threshold (Group B). For instance, in rats AA8 and AA12(VTA) there appears to be covariance of the required number and the maximum rate measures, evident at all currents, suggestive of a dependence of threshold on the value of maximum response rate. One may question to what extent the increases in required number represent an actual decrease in the rewarding impact of the stimulation. However, maximum response rates for these subjects recovered to baseline values whereas the required number remained elevated, above 0.1 $\log_{10}$ units in the case of AA8. Furthermore, in certain cases (e.g. AA13, AA21) decreases in maximum rates were obtained only after stabilization in the required number occurred. The most telling point remains that maximum response rates can vary independently from the threshold for self-stimulation.

It is generally assumed that once neurones within the central nervous system (CNS) die, they are never replaced. Nevertheless, the CNS displays an unexpected degree of plasticity in response to trauma. Furthermore, following a lesion, widespread biochemical and structural changes are

evoked elsewhere in the nervous system and may be important in the recovery of function. Therefore, transient effects, leading to partial improvement or complete recovery cannot be unambiguously interpreted, but by the same token cannot be dismissed as unimportant.

Since lesions can permanently affect the rewarding impact of the stimulation, as this and other studies have shown, transient effects tend to be somewhat overlooked. They nonetheless deserve some attentior because in a redundant system, or a system in which recovery of function is rapid, immediate effects may still indicate important, sought-after areas. The following example illustrates this point. In the visual system, cortical neurones respond selectively to prominent features of the visual world. The middle temporal area in the rhesus monkey (area MT or V5) is thought to contain neurones responsible for the perception of motion. Newsome and Paré (1986) produced partial lesions in this area and found that they cause a selective impairment in a motion discrimination task, which recovered to normal levels after a week. Nevertheless, Salzman, Britten and Newsome (1990) influenced perceptual judgments of motion by manipulating the discharge of directionally selective neurones within the MT area, with electrical microstimulation. Focal microstimulation selectively enhanced the neural signal related to a particular direction of motion and the monkey responded to this signal in a

meaningful way in the context of the experiment's behavioural paradigm. Thus, despite the transient effect of the lesions signifying a possible recovery process, it is now clear that cells in area MT directly influence motion perception.

Interpretation of Histological Findings

Effective Lesions

As the histology suggests, the lesion sites can be classified into two categories depending on the dorso-ventral location of the damage. Ventrally-placed lesions destroyed areas that include the MFB proper, including the LPOA, SI, ALH and LH. All 5 rats with the largest postlesion threshold increases belong in this category, as well as 2 of the 7 rats with transient increases in the required number threshold. The lesion sites for the remaining rats showing transient effects were located in the IC, the tissue surrounding the IC, and the structures medial to the IC, dorsal to the fibres of the MFB. Recently, Murray (unpublished doctoral dissertation) recorded from areas belonging in both categories, such as the SI, SM and IC and found cells with properties matching those psychophysically derived for first-order neurones. In sum, the histological findings support the view that structures rostral to the LH might be involved in MFB self-stimulation and raise a question as to the possible role that structures located dorsally to the MFB

might play in that phenomenon.

The relatively small size of many of the lesions in this study is curious, as is the inconsistency in the amount of damage produced across rats. Lesions that primarily damaged structures around the IC tend to be smaller than the ones centred along the anterolateral MFB. It is possible that the NMDA injections placed in the IC spread within this fibre bundle (Winn, personal communication)), thus producing less damage to the surrounding areas. Furthermore, evidence from quantitative autoradiography has shown that the NMDA receptors have a relatively low density in basal forebrain regions such as the GP, SI, BST, and thalamic regions such as the LH, preoptic area, and ZI (Monaghan & Cotman, 1985). Together, the unknown course of diffusion combined with the low density of NMDA receptors at the injection site might account for the small size of the lesions, as well as the inconsistencies observed across rats.

Ineffective lesions

Only three of the subjects in this study failed to show any changes in the required number, and one of these did not seem to have any damage (AA10). The regions of damage produced by the effective and ineffective lesions in this study were quite similar. Thus, it is difficult to attribute any differences in the effectiveness of the lesion based on

the histological observations. It is possible that these differences can be accounted for by inaccurate histological reconstructions, where the extent of the damage does not exactly correspond to the damage actually produced in the brain. Indeed, the data are presented as hand-made drawings, transferred using one's best judgment onto plates of an atlas. This is done instead of providing an exact representation of the actual tissue section, sacrificing precision for the ability to compare between subjects and across different studies. However, the transfer can be particularly imprecise in the representation of excitotoxic damage because of uncertainties regarding cell loss boundaries. It is the case that not all cells within the damaged area are killed. Furthermore, the differences between the relative placement of the lesion to the electrode tip between subjects could account for similar lesions yielding opposite effects.

One could claim that the introduction of fluid into the brain, especially at sites near the tip of the stimulating electrode might result in movement of the stimulated substrate with respect to the electrode. Studies using movable electrodes have shown that slight displacements can sometimes produce a large effect in the rewarding impact of the stimulation (Forgie & Shizgal, 1993, Rompré & Miliaressis, 1985). Thus, the possibility that any postlesion increase in required number is not due to the

lesion, cannot be ruled out. In that light, performing sham lesions where NMDA is replaced with the vehicle in which NMDA is dissolved, serves as an important control. In the 3 control rats used in this study, there were no changes in the required number from baseline following the lesion. Nevertheless, these controls do not eliminate the possibility that swelling consequent to tissue changes might occur after the NMDA lesions, displacing the reward substrate with respect to the electrode.

The Problem of Demyelination and nonspecific damage

The present experiment was carried out using neurotoxic lesions instead of the more widely used electrolytic lesions. The advantages of excitotoxic lesions have been stressed already in this thesis, but there are undoubtedly many aspects that need to be further explored. The complexity of interpreting excitotoxin lesions is accentuated by demyelination and nonspecific damage (holes), since they could well be responsible for the results obtained. For example, if demyelination occurs in the ventral thalamus, cell loss occurs in the anterolateral MFB, and the rate-frequency curves shift to the right, it is remotely possible that the curve-shifts might result from decreased conduction in the ventral thalamus. In this study, holes were observed in the area immediately below the injector tip. Methodological modifications, whereby the guiding cannula was

placed more dorsally in the brain, and the obturator was removed prior to perfusion, eliminated this problem. Thus, it is believed that these holes might not have been present prior to the histological preparation. This explanation cannot account for the case of AA8, where many distinct holes were present, enveloped by areas of cell loss and demyelination.

Surprisingly, the observed area of demyelination was smaller than that observed in the Stellar et al. study (1990). The demyelination was not consistently placed in the same areas in rats where the injection sites were relatively similar. The seemingly random location of demyelination casts doubts on the possibility that the demyelinated areas are responsible for the observed effects. Nevertheless, this randomness renders unfeasible experiments aimed at controlling for the possibility that the demyelinated area is in fact responsible for the observed effects. If, for similar injection sites, there existed a common demyelination area in all rats, electrolytic lesions in this area could reveal its relative importance in BSR. In the case of AA37 and AA39, where cell loss extended to the same areas, diametrically opposed patterns of demyelination emerged. In the case of AA39, absolutely no demyelination was observed while AA37 exhibited the largest amount of demyelination, covering the entire LPO and SI.

Redundancy of Function: Advantage of Excitotoxin Lesions

Given the large size of NMDA-produced lesions, it is difficult to determine which specific area is responsible for the effects. This, nevertheless, is not necessarily a hindrance in the application of excitotoxins as a lesioning tool. Many previous studies have shown that small, well-localised three-dimensional electrolytic lesions or other large, but two-dimensional knife-cut lesions failed to produce substantial increases in the required number. One possible explanation of these disappointing results is that neurones of different origins may be important for self-stimulation. It has been the cornerstone of the lesion methodology that the nervous system is organized in such a way as to display a high degree of localization of functions, with the localizable functions corresponding to known aspects of behaviour. Lesions have actually proven that there exist localization of function within areas such as the sensory or the visual cortex. Nevertheless, function need not be a direct consequence of a particular anatomically isolated group of cells. Functional redundancy in the reward system might be as logically useful as the modern notion of multiple memory systems, constructed to explain specific memory deficits as well as residual memory capacity after focal brain lesions in areas known to be involved in learning and memory. If first-order neurones are spread in a relatively large area of the brain, large lesions spreading in all three

planes might be required to produce long-term, substantial behavioural changes. In that light, the large size of NMDA lesions should be regarded as an auspicious procedural modification addressing an important theoretical concern.

Two pieces of evidence support the notion that large lesions might prove more effective than smaller ones. Firstly, the knife-cuts most effective in degrading stimulation reward efficacy in Waraczynski's (1988) study tended to be preoptic cuts, in which the damage produced an infection around the cut that invaded neighbouring tissue, giving it a three-dimensional configuration. Moreover, the effective lesions in the Murray and Shizgal (1991) study were produced by multistage electrolytic lesions that in most likelihood, increased the lesion volume. In some instances, a second or even a third lesion was required to alter the rewarding effect. In an attempt to extend the previous study, while maintaining histological accuracy by only producing one-step lesions, Murray (unpublished doctoral dissertation) was less successful in producing lesions of the anterior MFB capable of inducing changes in the reward system. It should be noted that this discussion contradicts the conclusion reached by Murray and Shizgal (1991) who emphasize that their results might be due to subtle differences in the anatomical location of the effective versus the noneffective lesions within a restricted area, such as the ALH.

There is yet another reason favouring large lesions over small ones. The fact that a particular behaviour is maintained following a lesion, is by itself, an important finding, potentially equally interesting to the loss of that behaviour. Persistence of behaviour after lesioning one part of the brain might actually strengthen inferences made on the basis of successful lesions in other parts. Indeed, large lesions have an edge over small ones insofar as they speed up both the discovery of candidate regions whose destruction affects behaviour, and the rejection of other regions whose removal points to the fact that the surviving structures are adequate for the expression of the behaviour.

A suggestion was put forward by Stellar et al. (1991) that within the framework of redundancy of function, it might prove beneficial to perform multiple, perhaps serial, lesions of different candidate sources of MFB fibres. The rationale for such an approach is well described by Marshall (1985): "After damage to brain region A, a behaviour is initially impaired and subsequently recovers. When region B is damaged concurrently with or subsequent to area A injury, this behaviour is irreversibly lost; however, damage to region B alone fails to produce the deficit." Excitotoxin lesions might set the stage for such an approach, inasmuch as within the large area of cell loss (that resulted in a big effect), there exist numerous nuclei whose role in the rewarding effect could be probed individually using the multilesion

approach. A compilation of histological descriptions of large lesions aimed at diverse regions could provide us with a global view of brain damage resulting in persistence of behaviour, as well as pinpoint regions of interest, as is the case with lesions that subsume some common area, but, nevertheless, have entirely different borders. The present data, for example, suggest the LPOA, the ALH, and the SI as nuclei that contain neurones contributing to the rewarding effect of stimulating more posterior MFB sites.

One argument against large lesions is that they might damage both agonistic and antagonistic systems, thus making it hard to discern any behavioural effect. In some cases, such as those reported by Waraczynski (1988), rats with extensive knife-cut transections of forebrain nuclei rostral to the LH stimulating electrode showed an <u>increase</u> in reward effectiveness. These data can be explained by hypothesizing a system in which both reward inhibition and reward excitation account for the final behavioural output. The lesions may have damaged more antagonistic than agonistic fibres and thus the final output will reveal increases in the rewarding effect of the stimulation. Nevertheless, it is not known how widespread such compensatory effects are. Another argument against large lesions is that they have been already tried with little success. Not taking into consideration older studies that did not employ curve-shift scaling, there are two studies that have examined the effects of large

lesions on self-stimulation. Colle and Wise (1987) found that large forebrain ablations resulted in substantial, sometimes transient increases, about 0.15 $\log_{10}$ units, in frequency threshold for several subjects. The magnitude of these effects is rather modest in relation to the amount of ablated tissue. Nevertheless, the ALH and regions dorso-lateral to it were not lesioned in this study. If these spared areas are important constituents of the BSR circuitry and furthermore, if the removal of several areas is necessary in order to see a large effect in reward efficacy, the modest threshold increases after only partial removal of the circuitry should come as no surprise. It is possible that removal of more than one of the candidate sources of the directly stimulated substrate, for example, the synergistic removal of the LPOA and the ALH, is needed to yield large impairments in reward. Separately removing each of these areas may result in only small and/or transient changes in rewarding efficacy. Stellar et al. (1991) made excitotoxin lesions aimed at the LH intrinsic neurones and concluded that the resulting lesions were ineffective. The criterion set by Stellar et al.(1991) for assessing the effectiveness of the lesions on the required number was larger than the 0.1 $\log_{10}$ units criterion used for Group B in this study. A closer examination of their data reveals that certain subjects showed some increases in the required number, from 0.1-0.16 $\log_{10}$ units, without demyelination affecting the electrode tip. Nevertheless, adoption of a more stringent criterion in

the present study would not affect the conclusions pertaining to subjects in Group A. The relative success of the present study could be attributed to the simultaneous destruction of regions that were separately lesioned in the aforementioned studies.

Dependence of Lesion Efficacy on the Stimulation Current

Alignment between the area of the lesion and the stimulated substrate has become an important issue in BSR. In fact, incidental misalignment is put forward as one of the reasons explaining the failure of much of the past lesion studies to find reward degradation. Misalignment could be the reason why small differences in the location of lesions have resulted in large differences in lesion effectiveness. The use of multiple currents was implemented to solve the problem of aligning the stimulated neurones with the lesion. The assumption behind this approach is that successively higher currents increase the cross-sectional area of activation. If the lesion of reward relevant cells is aligned with the tip of the electrode, we would expect to see reward degradation at low currents. If on the other hand the lesion is misaligned with the electrode tip, postlesion effects would only become evident at higher currents. In a lesion study with a large number of rats it would be reasonable to expect finding postlesion increases in the required number evident at both low and high currents.

Surprisingly, this prediction was not validated. All of the subjects in this study exhibited a uniform current dependency, with the largest increases in threshold evident at the lowest current and the smallest ones evident at the highest current. Does this mean that the stimulated neurones and the lesion were always aligned? One possibility is that the large area NMDA lesions occupy increases substantially the chances of damaging neurones projecting close to the electrode tip. Alternatively, the increased sensitivity at low currents could be an artifact due to the violation of assumptions pertaining to the counter model of spatiotemporal integration.

According to the counter model, the magnitude of the reward is determined by the total number of action potentials, regardless of their spatiotemporal distribution, that reach the network of neurones and synapses responsible for the summation of first-order neurone activity. In principle, the production of a set number of action potentials can be achieved by trading-off frequency and current in any imaginable combination of relative strengths. In practice however, the inverse proportionality between current and pulse frequency is not maintained at all stimulation parameters. For example, there is a lower limit, the current wall, under which self-stimulation cannot be obtained at any pulse frequency. Clearly, self-stimulation cannot be supported by high frequency stimulation of a single

axon. An obvious explanation pertains to the physiological limit on the frequency-following capacity of axons. In addition, as of yet unidentified constraints in the workings of the integrator might impose certain thresholds that stimulation parameters must have in order to produce a rewarding effect. In sum, it is important to recognize that the counter model breaks down at extreme stimulation parameters.

The low currents used in this experiment come close to such extreme parameters. That is, inverse proportionality between current and pulse frequency might not hold if the current is further reduced or, in an equivalent manner, if a lesion reduces the number of activated reward-relevant axons. To illustrate the consequences of the counter model breakdown in the postlesion required number, let us assume that the lesion removed half of the neurones carrying the reward signal, corresponding to halving the current. If the reward threshold at a current of, say, 400 uA was 80 Hz before the lesion, after the lesion, a doubling of the frequency to 160 Hz, (equal to the prelesion threshold at 200 uA), will be required to maintain reward magnitude at threshold. Nonetheless, the threshold at 200 uA might increase beyond a doubling after the lesion. If for instance, stimulation of 320 Hz manages to generate only 250 action potentials due to some conduction failure, further increases in frequency will be required to bring the reward magnitude at threshold.

Thus, it is likely that the threshold pulse frequency obtained at currents approaching the current wall is to a certain degree inflated. Such an effect would also increase the dynamic interval and could decrease the maximum rate

Indirect support for the notion that the low currents were near the current wall comes from the maximum rate analysis. Gallistel, Leon, Waraczynski, & Hanau (1991) demonstrated the empirically much observed finding that at low currents, close to the current wall, maximum rates are depressed in comparison to the ones obtained at higher currents. As may be seen for subject AA11, for example, lesioning decreased the maximum rate at the low current. This kind of asymptotic response decrement as a consequence of lesioning, restricted only at the lower current, suggests that the removal of reward-relevant fibres diminished the excitation produced before the lesion by the low current to that produced by the actual current wall.

Taken together, these changes can account for the largest increase in threshold observed at the lowest current tested. However, they are inadequate to account for larger lesion effects being obtained at the middle current in relation to those obtained at the high current. This is a perplexing problem with no obvious solution.

The solution to the problem of testing at low currents

approaching the current wall, where postlesion increases in the required number can be artificially inflated, would be to estimate the current wall before and after the lesion, by collecting rate-current curves at different values of high pulse frequencies (Waraczynski, et al., 1992). If the lesion removes reward-relevant fibres contributing to the stimulation-bound behaviour, then we would expect an increase in the required current. This increase would be proportional to the number of fibres that have been destroyed. In this case, increasing the current instead of the number of pulses provides a more accurate picture of the magnitude of the lesion effect.

Lack of Postlesion Effects for Stimulation of the VTA

Although it is clear from collision experiments that the LH and the VTA are linked by reward-relevant neurones, no major postlesion increase in the required number was observed for stimulation at the VTA sites. Only one animal (AA12) had substantial transient shifts for stimulation of both the LH and the VTA. Of the five rats with large increases in threshold at the LH, two responded for VTA stimulation. Inspection of the postlesion data in both rats indicates that thresholds were elevated from baseline, but only by less than 0.1 $\log_{10}$ units. To some extent the lack of VTA effects may be due to the smaller sample of animals that self-stimulated on the VTA as compared to those that self-stimulated on the

LH. Only half of the subjects (6 out of 12) with either large, or substantial, lesion effects for stimulation delivered through the LH electrode also responded for VTA stimulation. The relative lack of effects for stimulation of the VTA demands further discussion.

If one accepts the likelihood that stimulation of the MFB recruits more than one population of first-order neurones, it is natural to ask whether the lack of VTA effects is due to the differential activation of neuronal populations that do not summate their outputs. As may be seen in Fig. 36, it is possible to conceive of an anatomical arrangement with two reward-relevant neuronal populations, one passing through both the LH and the VTA and the other coursing only through the VTA. If the outputs of the two populations do not summate so that stimulation of both populations is as effective as stimulation of the more dominant population, a lesion affecting the cells giving rise to the fibres common to the two sites will produce a substantial increase in threshold only for stimulation of the LH. The effect at the VTA will be much smaller, if any, since the population that remains can support self-stimulation.

Future directions

The primary objective of the present work was to explore

Fig. 36. Diagram illustrating the multiple-system hypothesis of MFB self-stimulation. The LH and the VTA are connected by a common system of reward-relevant fibres. A separate system courses through the VTA. Assuming poor or no summation between the two systems, a lesion (filled circle) rostral to the LH is likely to degrade the reward impact of the LH self-stimulation but would have little or no effect on VTA self-stimulation.


LH
VTA

the possibility that the basal forebrain contains nuclei that give rise to the first-order neurones. In general, NMDA lesions in that region reduced the rewarding impact of stimulating more caudal sites along the MFB. However, the problems of nonspecific damage and demyelination do not allow for unambiguous interpretation of these results. Procedural modifications have significantly reduced the loss of tissue integrity, but demyelination remains a cause for concern. Thus, a primary focus of future studies should be to devise approaches with the potential to circumvent the problem of demyelination.

Initially, hopes were raised that remyelination might be a sufficient solution. If remyelination occurred some time after the induction of the lesion and the approximate time course of myelin regeneration was established, then behavioural effects obtained after the remyelination would be attributed solely to cell-body loss. Remyelination can occur on demyelinated central nerve fibres including those in the plaque of multiple sclerosis (Prineas, 1985; Raine, Moore, Hintzen, & Traugott, 1988) and in those experimentally induced in animals (Herndon, Price, & Weiner, 1977; Ludwin, 1978). Furthermore, remyelinated fibres were shown to conduct normally after remyelination in an experimental demyelinating lesion of the cat spinal cord (Smith, Blakemore, & McDonald, 1981). An increase of sodium channels along the demyelinated areas, such as the one seen in human

multiple sclerosis lesions (Moll, Mourre, Lazdunski, & Ulrich, 1991), might contribute to the restoration of conduction. Recently, Brace, Latimer, and Winn (1992) provided evidence of remyelination in the rat LH consequent to NMDA-induced lesions. Nevertheless, even four months after the lesion remyelination was still incomplete. The relevance of these findings remains unclear in the absence of electrophysiological studies showing recovery of conduction through the lesioned area. The present data show that demyelination can persist after three months of postlesion testing (e.g. AA37). Thus, waiting for remyelination to occur does not seem to be the preferred solution to the problem of demyelination. The prevention of demyelination becomes a priority.

Coffey et al. (1988) suggested that demyelination is due to the inflammatory response and the recruitment of immune cells, such as macrophages, in the area of the lesion. Presumably, infiltration is facilitated by the breakdown of the blood-brain barrier which becomes evident in the days following the lesion (Brace et al., 1992; Marty, Dusart, & Peschanski, 1991). Also involved in the inflammatory response are microglia which constitute the predominant glial cell type in the neurone-depleted area over the first weeks following the lesion (Giulian, 1987; Marty et al., 1991). Astrocytes form the classical glial scar only after a month from the initial damage (Dusart, Marty, & Peschanski, 1991).

Assuming that the nonspecific outcome of excitotoxin-induced cell death, the activation of microglia and the infiltration of blood-derived macrophages, is responsible for demyelination, antiinflammatory treatment could be of some benefit in at least reducing the demyelination. To that effect, possible intervention includes administration of steroids such as dexamethasone or more potent immunosuppressants such as cyclophosphamide. Alternatively, using experimental autoimmune encephalomyelitis (EAE - an inflammatory condition characterized by demyelination of the CNS) as a model, Huitinga, Van Rooijen, De Groot, Ultdehaag, and Dijkstra (1990) suppressed demyelination by eliminating macrophages from the circulation and from the brain. Elimination of macrophages was achieved by intravenous injection of mannosylated dichloromethylene-di-phosphonate containing liposomes, that are capable of crossing the blood-brain barrier.

Yednock, Cannon, Fritz, Sanchez-Madrid, Steinman, and Karin (1992) showed that blocking the endothelium adhesion molecules, which mediate the recruitment of leukocytes, with monoclonal antibodies, prevented EAE. Despite the methodological difficulties associated with the development of monoclonal antibodies, blocking leucocyte entry into the CNS after the excitotoxin lesion with antibodies directed against the selectins and the integrins should be considered as a treatment of demyelination. This method's specificity

renders long-term testing possible, an absolute requirement of behavioural studies. Its application would not lead to a general reduction of the immune response, hence, would not leave animals vulnerable to infection.

The use of proteinase inhibitors has been suggested as a treatment for demyelinating diseases (Banik, 1992). High levels of proteolytic enzymes are found in a variety of diseases involving myelin breakdown. These enzymes are manufactured by immune cells following their activation and probably play a role in clearing the damaged area from debris. Altering the activity of those enzymes is likely to prevent demyelination. Indeed, the development of EAE was suppressed by daily administration of the inhibitors epsilon aminocaproic acid and aminomethyl cyclohexane carboxylic acid (Brosnan, Cammer, Norton, & Bloom, 1980).

If the approach of restricting immunoreactivity fails, myelin-destructive drugs such as ethidium bromide or l-a-lysophosphatidyl-choline, or antibodies against myelin could be injected before the excitotoxic lesion, during the collection of baseline data, to create a baseline where demyelination would no longer be a confounding factor for the interpretation of the postlesion effects. Moreover, it would be informative to inject these demyelinating agents alone to test the descending path hypothesis. Provided that the behaviourally relevant direction of conduction is rostro-

caudal, application of a demyelinating agent rostrally to the stimulated sites would not be expected to alter the efficacy of the stimulation or the behaviourally derived estimates of refractory periods. In contrast, injections caudal to the stimulating electrodes should increase the thresholds for self-stimulation and change the refractory period estimates. Continuing evaluation of the descending path hypothesis is crucial for the shaping of future experiments aimed at the discovery of the transducer cells in MFB stimulation reward.

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