THE POSTNATAL DEVELOPMENT OF POST-ACTIVATION POTENTIATION IN THE NEOCORTEX AND DENTATE GYRUS OF THE RAT

By

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ABSTRACT

The postnatal development of short-term potentiation (STP) and long-term potentiation (LTP) were examined in the neocortex and dentate gyrus of the rat. It was found that STP and LTP develop during narrowly defined time periods in both systems. This functional synaptic development did not appear to correspond well with known structural development in either system (e.g. synaptogenesis or dendritic spine formation).

Stimulation of callosal fibers produced a biphasic, positive-negative, transcallosal response (TCR) recorded near the surface of the anterior neocortex in all ages tested. The TCR showed a decrease in threshold, latency and halfwidth, and an increase in peak amplitude with age. STP and LTP of the TCR could not be reliably detected until after PN16 and PN18, respectively. The magnitude of STP and LTP was initially small but approached adult levels rapidly after their initial appearance.

Stimulation of perforant path fibers produced a positive excitatory post-synaptic potential (EPSP) with a super-imposed negative population spike recorded in the dentate hilus. The EPSP showed a decrease in threshold, latency and halfwidth, and an increase in peak amplitude with age. STP and LTP (of the EPSP and/or population spike) could not be reliably detected until the second postnatal week, with STP appearing prior to LTP. Again, STP and LTP approached adult levels rapidly after their initial appearance. These results could not be

explained by differential effects of anesthesia on immature animals, nor by higher STP/LTP thresholds in immature animals.

The maturation of evoked response morphology (e.g. threshold and latency) did not correspond closely with STP/LTP development in either the hippocampal or neocortical system. Also, the correspondence between STP/LTP development and structural developments such as synaptogenesis, spine formation, or myelinogenesis (as reported in the literature) was not particularly strong in either system.

These results suggest that the postnatal development of STP and LTP, and thus the mechanism of potentiation effects in mature animals, may not depend so much on the maturation of specific structures (e.g. dendritic spines) as on the maturation of neurochemical processes (e.g. receptor or protease development). Possible PAP-mechanisms were discussed.

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ABBREVIATIONS

AD	After-discharge
aTCR	anterior Trans-callosal response
CNS	Central nervous system
EPSP	Excitatory post-synaptic potential
GABA	Gamma amino butyric acid
I/O	Input/Output
IPI	Inter-pulse interval
IPSP	Inhibitory post-synaptic potential
LTP	Long-term potentiation
MEPP	Miniature end plate potential
NMJ	Neuromuscular junction
PAP	Post-activation potentiation
PN	Postnatal
PNS	Peripheral nervous system
pTCR	posterior Trans-callosal response
PTP	Post-tetanic potentiation
STP	Short-term potentiation

CHAPTER I. INTRODUCTION

It is commonly believed that functional changes at the synapse underlie memory formation (e.g., Kandel & Spencer, 1968; Hebb, 1949). Many specific hypotheses have been proposed that account for learning and memory phenomena by increases or decreases in synaptic efficacy (Fillenz, 1972; Gardner-Medwin, 1969; Gilbert, 1975; Goddard, 1980; Griffith, 1966; Hebb, 1949; Rosenzweig, Mollgaard, Diamond & Bennett, 1972; Schuz, 1978; Willshaw, Buneman & Longuet-Higgins, 1969). Any model of the synaptic changes underlying learning and memory processes must account for rapidly occurring, yet long-lasting, changes in synaptic function following physiologically plausible events.

One model that satisfies these criteria is post-activation potentiation (PAP). PAP is an increase in synaptic efficacy following brief periods of repeated, patterned synaptic activation. PAP has been reported to occur at synapses in the peripheral nervous system (PNS) (Hughes, 1958; Magleby & Zengel, 1976a; Magleby & Zengel, 1976b; Zucker, 1974) as well as the central nervous system (CNS) (Bishop, Burke & Hayhow, 1959; Eccles & McIntyre, 1953; Lomo, 1971; Bliss & Lomo, 1973, Douglas & Goddard, 1975; Alger & Teyler, 1976; Andersen, Sundberg, Sveen & Wigstrom, 1977; Gerren & Weinberger, 1983; Komatsu, Toyama, Maeda & Sakaguchi, 1981; Lee, 1982; McNaughton, Douglas & Goddard, 1978; Misgeld, Sarvey & Klee, 1979; Racine & Milgram, 1983; Racine, Milgram & Hafner, 1983; Teyler, Alger, Bergman & Livingston, 1977; Wilson &

Racine, 1981; Wilson & Racine, 1983). At some central synapses, PAP can be induced by brief periods of high frequency stimulation (8 pulses at 400 Hz) and can last for days or weeks (Bliss & Gardner-Medwin, 1973; Barnes, 1979; Douglas & Goddard, 1975; Racine, et al., 1983). Although PAP has been well described in several forebrain pathways, the mechanism through which synaptic activation leads to an increase in synaptic efficacy in the CNS, is not yet known (Bliss, 1979; Swanson, Teyler & Thompson, 1982).

The present thesis describes the postnatal development of PAP in the CNS of the rat. A correlation of PAP development with specific neural structural or neurochemical developments may provide some insight into the mechanisms of PAP in the mature animal. During the ontogeny of any given neural system, however, many events occur simultaneously. Singling out one correlate of PAP development in a single system, may be only suggestive at best, and misleading at worst. One way to strengthen this developmental approach would be to examine two neural systems that develop at different times or rates. Thus, if a certain developmental correlate of PAP in one system is indeed important to PAP ontogeny, that correlation should exist in both systems examined. This was the approach used here.

The two systems chosen for this study were the perforant path input to the dentate gyrus and the intrinsic callosal pathway in the neocortex. These systems were chosen for several reasons. Most research done on PAP in the mature CNS, in vivo, has been done in the dentate gyrus (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973; Douglas & Goddard, 1975; McNaughton, et al., 1978; Douglas, 1977; Barnes, 1979;

Robinson & Racine, 1982; Bliss, Goddard & Riives, 1983; Wilson, 1981; Wilson, Levy & Steward, 1981). The dentate gyrus is a part of the hippocampal formation, which has been strongly linked to memory processes (Douglas, 1967; O'Keefe & Conway, 1980; O'Keefe & Nadel, 1978; Olton, Becker & Handelmann, 1980; Scoville & Milner, 1957). In addition, the hippocampus has a unique cytoarchitecture (to be discussed below) that lends itself well to electrophysiological analysis. The neocortex, while not as electrophysiologically "simple" as the hippocampal formation, has long been considered important in memory (e.g., Pavlov, 1927; Sechenov, 1863), and has been shown to be capable of several forms of structural and functional plasticity (Lippold, 1970; Hubel & Wiesel, 1962; Hubel & Wiesel, 1970; Rothblat & Schwartz, 1979; Valverde, 1967; Yinon & Auerbach, 1973; Clare, Landau & Bishop, 1961). Although a detailed study of neocortical PAP has not yet been done, there is evidence that neocortical synapses can demonstrate PAP (Clare, et al., 1961, Komatsu, et al., 1981; Lee, 1982; Racine, Tuff & Zaide, 1975).

Both the neocortex and hippocampus in the rat undergo major postnatal maturational changes in anatomy (Aghajanian & Bloom, 1967; Altman & Das, 1965; Cotman, Taylor & Lynch, 1973; Crain, Cotman, Taylor & Lynch, 1973; Duffy & Teyler, 1978; Johnson & Armstrong-James, 1970; Loy, Lynch & Cotman, 1977; Schlessinger, Cowan & Gottlieb, 1975; Schwartzkroin, Kunkel & Mathers, 1982; Seggie & Berry, 1972; Wise, Fleshman & Jones, 1979; Wise & Jones, 1978) neurochemistry, (Baudry, Arst, Oliver & Lynch, 1981; Coyle & Enna, 1976; Coyle & Yamamura, 1976; Matthews, Nadler, Lynch & Cotman, 1974; Sanderson & Murphy, 1982;

Nadler, Matthews, Cotman & Lynch, 1974) and physiology (Armstrong-James & Williams, 1963; Deza & Eidelberg, 1967; Grafstein, 1963; Purpura, Prelevic & Santini, 1968; Purpura, Shofer & Scarff, 1965; Shofer & Purpura, 1972; Schwartzkroin, 1982; Schwartzkroin & Altschuler, 1977; Seggie & Berry, 1972). These structures are still very immature at birth in the rat which allows monitoring of maturational events postnatally.

Finally, in many aspects, hippocampal maturation precedes neocortical maturation. The fact that they mature at different times will allow some separation of meaningful, from coincidental, developmental correlates of PAP, as discussed above. The details of the postnatal development of these structures will be given below.

I. A. POST-ACTIVATION POTENTIATION

Post-activation potentiation is an increase in synaptic efficacy following repetitive synaptic activation, and has been described in several mature forebrain pathways (Alger & Teyler, 1976; Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973; Douglas & Goddard, 1975; Lee, 1982; Racine & Milgram,1983; Racine, et al., 1983; Wilson & Racine, 1981). In many forebrain pathways, PAP can be described by at least three major components; facilitation, lasting less than 1 second (Creager, Dunwiddie & Lynch, 1980; Lomo, 1971b; Racine & Milgram, 1983), short-term potentiation (STP), lasting several minutes (McNaughton, 1980; Racine & Milgram, 1983), and long-term potentiation (LTP), lasting up to hours, days or weeks (Bliss & Lomo, 1973; Douglas & Goddard, 1975; Racine, et al., 1983).

Short-term post-activation potentiation: The transient forms of PAP, facilitation and STP, have been described in greatest detail in the peripheral nervous system, in particular at the neuromuscular junction (n.m.j.) (for a review see Hughes, 1958). The study of PAP at the n.m.j. allowed the activity of a single synapse to be examined and manipulated. Although the ionic mechanisms of short-term PAP have not been examined in the CNS as thoroughly as at the n.m.j., PAP phenomena are similar at both sites and similar mechanisms are thought to be involved.

The shortest duration PAP phenomenon is facilitation, an increase in test response amplitude following a single conditioning pulse. The amplitude of the test response can be increased 100-200% above conditioning response amplitude at the n.m.j. (Mallart & Martin, 1967; Mallart & Martin, 1968; Zucker, 1974) and in the forebrain (Creager, et al., 1980; Lomo, 1971b; Racine & Milgram, 1983; White, Nadler & Cotman, 1979). The decay of facilitation at the n.m.j. can be described as the sum of two components, with time constants of 35 msec and 120 msec (Mallart & Martin, 1967). In pathways examined in the rat forebrain, facilitation decays exponentially, with a time constant of 80-90 msec (Creager, et al., 1980; Racine & Milgram, 1983). At the n.m.j., the mechanism of facilitation appears to be presynaptic. The conditioning impulse causes a transient increase in pre-synaptic Ca^{++} concentration, which in turn causes an increased number of transmitter quanta to be released in response to subsequent test pulses (Del Castilo & Katz, 1954; Mallart & Martin, 1967; Zucker, 1974).

STP (also known as post-tetanic potentiation, PTP) involves an increase in test response amplitude following high frequency synaptic activation. At the n.m.j., an increase in the frequency of spontaneous miniature end-plate potentials (mepp's) is also seen following the application of conditioning trains (Lev-Tov & Rahamimoff, 1980; Liley, 1956; Weinreich, 1971). The magnitude and duration of STP at the n.m.j. is dependent on the frequency and duration of the conditioning train (Lev-Tov & Rahamimoff, 1980; Liley, 1956; Magleby & Zengel, 1975; Magleby & Zengel, 1976a; Zengel & Magleby, 1982; Rosenthal, 1969; Weinreich, 1971). Train intensity also affects potentiation, at least in forebrain pathways (McNaughton, 1980; McNaughton, 1982).

STP can be divided into two distinct components at the n.m.j. (Magleby & Zengel, 1976a), with an additional, third component at many forebrain synapses (Racine & Milgram, 1983). These components decay exponentially and can be identified by their time constants of decay. Augmentation, in which response amplitude can be increased as much as 8 times baseline, decays at the n.m.j. with a time constant of 7 seconds (Magleby & Zengel, 1976a). A similar component has been described in the forebrain (McNaughton, 1980; Racine & Milgram, 1983). The magnitude of augmentation is dependent on the strength of the conditioning train, although its duration is constant (Magleby & Zengel, 1976a).

Potentiation, the second component of STP, has a time constant ranging from 20 seconds to several minutes, depending on the strength of the conditioning train (Lev-Tov & Rahamimoff, 1980; Liley, 1956; Magleby & Zengel, 1976a). Again, a similar component has been identified in the rat forebrain (McNaughton, 1980; Racine & Milgram, 1983). The magnitude

of potentiation is dependent on the strength of the conditioning train and on the synapse involved, but ranges up to several times baseline (Magleby & Zengel, 1976a, McNaughton, 1980; Racine & Milgram, 1983).

A third component of STP has recently been identified in the rat limbic forebrain, and labeled potentiation 2 (Racine & Milgram, 1983). Potentiation 2 is often seen in the absence of both augmentation and potentiation (1), and decays exponentially with a time constant of around 6.5 minutes (Racine & Milgram, 1983).

The differences in the time course and magnitude of augmentation, potentiation and potentiation 2, suggests different underlying mechanisms. Augmentation and potentiation have been shown to be Ca^{++} dependent, and involve an increase in transmitter output at the n.m.j. (Del Castilo & Katz, 1954; Lev-Tov & Rahamimoff, 1980; Rosenthal, 1969; Weinreich, 1971). It is hypothesized that high frequency stimulation of the n.m.j. results in an increase in presynaptic Ca^{++} concentration, resulting in a subsequent increase in spontaneous and evoked transmitter release (Magelby & Zengel, 1975; Magleby & Zengel, 1976a). Although this proposed mechanism for STP is very similar to that stated above for facilitation (Mallart & Martin, 1967), facilitation and STP interact multiplicatively at the n.m.j. under conditions of low quantal output (Magleby, 1973b). Furthermore, the magnitude and duration of STP are greater than would be predicted from a simple linear summation of facilitation following each pulse in a conditioning train (Magleby, 1973a). These results suggest that STP and facilitation probably rely on somewhat different underlying mechanisms (Landau, Smolinsky & Lass, 1973).

Long-term post-activation potentiation: LTP was initially discovered in the hippocampal formation (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973), and has since been studied in greatest detail in that structure (Alger & Teyler, 1976; Andersen, et al., 1977; Bliss, 1979; Bliss, 1982; Teyler, et al., 1977; Yamamoto & Chujo, 1978). Subsequent mapping studies indicate that several other forebrain pathways can also support LTP (Racine, et al., 1983, also see Eccles & McIntyre, 1953; Gerren & Weinberger, 1983; Brown & McAfee, 1983, for non-forebrain pathways that may support LTP). LTP can last several days or weeks (Barnes, 1979; Bliss & Gardner-Medwin, 1973; Douglas & Goddard, 1975; Racine, et al., 1983). The magnitude of LTP appears dependent on the pathway examined, with the greatest magnitude found in pathways leading into, out of, or intrinsic to, the hippocampal formation (Racine, et al., 1983). The decay of LTP appears best described by the sum of two exponentials, with time constants of 1.5 hours and 5 days (Racine, et al., 1983).

Most evidence currently favors a post-synaptic mechanism for LTP (Andersen, Sundberg, Sveen, Swann & Wigstrom, 1980; Baudry, Oliver, Creager, Wieraszko & Lynch, 1980; Bliss, et al., 1983; Deadwyler, Dunwiddie & Lynch, 1978; Desmond & Levy, 1983; Douglas, Goddard & Riives, 1982; Dunwiddie & Lynch, 1978; Dunwiddie, Madison & Lynch, 1978; Fifkova, Anderson, Young & Van Harreveld, 1982; Krug, Brodemann & Ott, 1982; Lee, Schottler, Oliver & Lynch, 1980; Levy & Steward, 1979; Lynch, Dunwiddie & Gribkoff, 1977; Lynch, Gribkoff & Deadwyler, 1976; Lynch, Halpain & Baudry, 1982; McNaughton, 1982; McNaughton, et al., 1978; Misgeld, Sarvey & Klee, 1979; Van Harreveld & Fifkova, 1975; Wigstrom &

Gustafsson, 1983; Wigstrom, McNaughton & Barnes, 1982; Wilson, 1981; Wilson, et al., 1981), although there is some evidence for pre-synaptic action as well (Baimbridge & Miller, 1981; Bliss & Dolphin, 1982; Dolphin, Errington & Bliss, 1982; Skrede & Malthe-Sorenssen, 1981; Turner, Baimbridge & Miller, 1982).

The evidence for a post-synaptic mechanism of LTP includes the following: 1) the induction of LTP requires transmitter activation of the post-synaptic cell (Dunwiddie, et al., 1978; Krug, et al., 1982). Blockade of post-synaptic transmitter receptors during the conditioning train blocks LTP; i.e., response amplitude returns to pre-train amplitude when the blocker is removed. 2) The induction of LTP requires coactivation of a minimal number of afferents (Levy & Steward, 1979; McNaughton, et al., 1978; Wilson, et al., 1979), suggesting a threshold post-synaptic response must be produced to induce LTP. 3) LTP is specific to the activated afferents in area CA1 and the dentate gyrus of the hippocampal formation (Andersen, et al., 1980; McNaughton & Barnes, 1977), but may be associated with heterosynaptic depression in area CA1 (Lynch, et al., 1977) and heterosynaptic potentiation in area CA3 (Misgeld, et al., 1979; Yamamoto & Chujo, 1978; Yamamoto, Matsumoto & Takagi, 1980). These heterosynaptic effects clearly suggest a post-synaptic change associated with LTP, since there is no evidence of pre-synaptic connections in the hippocampus. 4) Heterosynaptic interactions occur at several sites when the inputs are concurrent. Again this suggests a post-synaptic locus of control for LTP (Bliss, et al., 1983; Douglas, et al., 1982; Robinson & Racine, 1982). For example, activation of a strong inhibitory input to dentate granule cells prior

to conditioning of the excitatory input to the same cells prevented, or reduced, the amount of LTP observed at the excitatory synapses (Douglas, et al., 1982). Furthermore, simultaneous activation of two independent excitatory pathways converging onto a single postsynaptic population can produce greater LTP than identical activation of either pathway alone (McNaughton, et al., 1978). 5) Post-synaptic dendritic spines change in shape following LTP-producing activation of afferents impinging on them (Desmond & Levy, 1983; Lee, et al., 1980; Van Harreveld & Fifkova, 1975). A change in dendritic spine morphology could decrease spine resistance, increasing the synaptic current flow into the dendritic shaft (Bliss & Lomo, 1973). 6) Putative post-synaptic transmitter receptors are increased in number following LTP-producing activation of hippocampal pathways (Baudry, et al., 1980; Lynch, et al., 1982). 7) Intracellular injection of EGTA, a Ca^{++} chelator, into post-synaptic cells blocks LTP in those cells, while leaving STP and facilation unaffected (Lynch, Kelso, Barrionuevo, Larson & Schottler, submitted).

The major evidence for a pre-synaptic mechanism of LTP is an increase in resting and evoked transmitter release following high frequency activation (Dolphin, et al., 1982; Skrede & Málthe-Sorenssen, 1981). This increased transmitter output may be supported by an increased synaptic uptake and retention of Ca^{++} following high frequency activation of those synapses (Baimbridge & Miller, 1981).

I. B. FUNCTIONAL ANATOMY AND DEVELOPMENT OF THE NEOCORTEX

The mature rat neocortex is organized into six laminae oriented tangentially to the cortical surface. These laminae are differentiated by the type and density of cells present, and are identified by numbers I to VI, from the cortical surface to the underlying white matter, respectively. The cytoarchitecture of the mature neocortex is extremely complex and varies across cortical areas. As the experiments detailed in the following chapters involve the trans-callosal response, the following review will deal primarily with the anatomy and development of the corpus callosum. For reviews of the anatomy of the neocortex in general, see Colonnier (1981), Jones (1981a), and Szentagothai (1969).

Although the neocortex is composed of many diverse cell types (Szentagothai, 1969), pyramidal and stellate neurons are the primary projection neurons (Colonnier, 1981; Schwartz & Coleman, 1981) and receive most of the afferent terminations (Colonnier, 1981; Szenthagothai, 1969). The mature pyramidal cell consists of a cone shaped soma, a large, upward-projecting, spiny apical dendrite, short basal dendrites, and a downward-projecting myelinated axon. Large or giant pyramidal cells tend to be located in the deeper layers, Layers V and VI (Szentagothai, 1969). Smaller pyramidal cells are located in Layers II and III. Cortical pyramidal cells receive symmetrical synapses primarily on dendritic spines and asymmetric contacts on the soma and dendritic shaft (Colonnier, 1968). Symmetric synapses are thought to be excitatory and asymmetric synapses, inhibitory (for a review, see Colonnier, 1981). Cortical pyramidal output appears to be excitatory (Emson & Hunt, 1981; Wolff & Chronwall, 1982).

Stellate neurons are of varied morphology. The majority of stellates contain gamma-amino butyric acid (GABA) and form inhibitory synapses within the cortex (Emson & Hunt, 1981; Wolff & Chronwall, 1982). Spiny stellates are thought to be excitatory interneurons (Colonnier, 1981), and may receive direct extrinsic afferent input (White, 1981).

The corpus callosum projects from, and terminates within, discrete vertical strips up to 1 mm in width (Goldman & Nauta, 1977; Gould & Kaas, 1981; Heimer, Ebner & Nauta, 1967; Jacobsen, 1970; Jones, 1981a). Between these strips, in areas of sparse callosal terminations, are areas of dense thalamic input (Vogt, Rosene & Peters, 1981; Wise & Jones, 1978). Callosal and thalamic inputs are further separated intralaminarly, with thalamic terminations primarily in Layers I and IV (Herkenham, 1980; Wise & Jones, 1978), and callosal terminations in Layers I-III and VI (Jacobsen, 1970; Vogt, et al., 1981; Wise & Jones, 1978). Regional differences in the density of callosal terminations also exist, with, for example, the primary visual cortex having notably sparse callosal connections (Jacobsen, 1970; Cusick & Lund, 1982).

Callosal projection neurons are pyramidal cells located primarily though not exclusively, in Layers III and V (Ivy & Killackey, 1981). These neurons project to the homologous site in the contralateral hemisphere (Jones, 1981a). A single callosal fiber may branch within the cortex and terminate in several Layers (Hartenstein & Innocenti, 1981). Callosal fibers terminate on both pyramidal and non-pyramidal dendrites (Globus & Scheibel, 1967; Vogt & Gorman, 1982).

Although the neurotransmitter of callosal axons has not been identified, some evidence suggests it may be the amino acid glutamate. Pyramidal cells of Layers III and V, which are the callosal projection neurons (Ivy & Killackey, 1981), appear to use glutamate as their transmitter (Emson & Hunt, 1981).

An electrical pulse, applied to the corpus callosum, produces a biphasic, positive-negative potential, recorded at the surface of the opposite hemisphere (Chang, 1953; Clare, et al., 1961; Curtis, 1940a,b; Grafstein, 1959; Latimer & Kennedy, 1961; Seggie & Berry, 1972). This surface positive-negative trans-callosal response (TCR) reverses in potential as the recording electrode is lowered through the cortex (Clare, et al., 1961). The TCR coincides with an EPSP-IPSP sequence recorded in Layer V neurons, often preceded by an antidromic spike (Vogt & Gorman, 1982). Single unit activity is concentrated on the falling positivity-rising negativity phase of the TCR (Latimer & Kennedy, 1961).

Development: The rat neocortex is one of the final structures to complete development postnatally. The newborn rat cortex, in contrast to the mature cortex, is characterized by just two prominent layers; the cortical plate, a cell-dense, superficial layer, and the sub-plate, a less cell-dense layer (Ivy & Killackey, 1981). At birth, cells in the cortical plate appear bipolar, with few, if any basal dendrites, and a single, unbranching apical dendrite extending toward the cortical surface. Cells of the subplate have already attained the basic, although immature, pyramidal morphology (Ivy & Killackey, 1981). By postnatal day 4 (PN4), the cortical plate - sub-plate distinction begins to breakdown, with the bipolar cells of the cortical plate developing

into Layer II and III pyramidal cells. For the first several weeks, the morphological development of neurons in deeper layers precedes that of more superficial neurons (Berry, 1981).

Synaptogenesis begins around the date of birth (Johnson & Armstrong-James, 1970), with symmetric synapses forming on pyramidal dendrites by PN3 and asymmetric synapses by PN9 (Miller & Peters, 1981). Synaptogenesis continues, with a large increase during the third and fourth postnatal weeks, until adult levels are reached near PN35 (Aghajanian & Bloom, 1967).

The majority of afferent terminations in the mature neocortex are on dendritic spines (Colonnier, 1981). Dendritic spines first appear toward the end of the second postnatal week and show extensive increases in number (Wise, et al., 1979) and morphological complexity (Miller & Peters, 1981) over the following week. In addition to these changes in post-synaptic structure, the number of vesicles in pre-synaptic terminals increases following initial synaptic formation (Johnson & Armstrong-James, 1970; Jones, 1982).

Callosal fibers begin invading the contralateral neocortex on PN4-PN5 (Jones, 1981b) and reach their sites of termination near the end of the first postnatal week (Jones, 1981b, Wise & Jones, 1978). Myelination of these fibers begins around PN13 (Caley & Butler, 1974; Seggie & Berry, 1972).

The immature TCR has been studied in both the cat (Grafstein, 1963; Shofer & Purpura, 1972) and rat (Poon, 1965; Seggie & Berry, 1972). The TCR demonstrates a decrease in latency, and increase in amplitude with age (Grafstein, 1963; Poon, 1965; Seggie & Berry, 1972).

Results from intracellular studies of immature neocortical neurons are contradictory (Komatsu, 1983; Purpura, et al., 1965), however the most recent studies suggest a late development of inhibition (Komatsu, 1983). These recent intracellular results are in agreement with histological studies showing late development of asymmetric synapses (Miller & Peters, 1981).

I. C. FUNCTIONAL ANATOMY AND DEVELOPMENT OF THE DENTATE GYRUS

The dentate gyrus is a component of the hippocampal formation, and runs parallel to, and just below the hippocampal gyrus. The dentate's primary cell type is the granule cell, as opposed to the pyramidal cells of the hippocampal gyrus.

The mature dentate gyrus consists of a horse-shoe shaped band of closely packed granule cell bodies. This horse-shoe straddles the pyramidal cells of hippocampal region CA4, and thus can be divided into a suprapyramidal and infrapyramidal blade. The granule cells are oriented such that the apical dendritic tree extends away from the center of the formation. This dendritic region is called the molecular layer and is the primary region of afferent termination in the dentate. The granule cell axons project in the opposite direction from the dendrites, through the dentate hilus, or CA4. These axons, called mossy fibers, constitute the dentate's sole efferent projection. The mossy fibers make <u>en passage</u> contacts on the apical dendrites of hippocampal CA3 pyramidal cells (Andersen, Blackstad & Lomo, 1966).

The major afferents to the dentate gyrus come from the entorhinal cortex, septum and the contralateral hippocampus. Fibers

from catecholaminergic brain stem nuclei also terminate in the dentate (Loy, Koziell, Lindsey & Moore, 1980, Winson, 1980).

The ipsilateral entorhinal cortex projects to the dentate via the perforant path. The perforant path is composed of small diameter myelinated axons (Nafstad, 1967) from diverse cell types in Layers II and III of the entorhinal cortex (Schwartz & Coleman, 1981). The perforant path is divided into medial and lateral components depending on the location of the entorhinal projection cells (Hjorth-Simonsen, 1972; McNaughton, 1980; McNaughton & Barnes, 1977). Lateral perforant path fibers make en passage contacts on dendritic spines in the outer one-third of the molecular layer (Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972). Medial perforant path fibers make en passage contacts on the molecular layer medial one-third (Hjorth-Simonsen, 1972; Hjorth-Simonsen & Jeune, 1972). Terminations on the inner one-third are predominantly formed by septal and commisural fibers (Gottlieb & Cowan, 1973; Hjorth-Simonsen & Laurberg, 1977; Mosko, Lynch & Cotman, 1973).

In addition to granule cells, histological (Cowan, Stanfield & Amaral, 1981; Gottlieb & Cowan, 1973) and electrophysiological (Andersen, Holmqvist & Voorhoeve, 1966; Dudek, Deadwyler, Cotman & Lynch, 1976; Lomo, 1971a,b) evidence suggests the presence of inhibitory basket cells. These basket cells are generally located in, or below, the granule cell body layer and form inhibitory connections on the granule cells somata (Andersen, et al, 1966; Cowan, et al., 1981). The basket cells receive input from mossy fiber collaterals to form a recurrent inhibitory circuit. In addition, basket cells may send dendrites into the molecular layer to receive input directly from perforant path

fibers, thus forming a feed-forward inhibitory system (Cowan, et al., 1981).

Current evidence indicates that the amino acids glutamate or aspartate may be the neurotransmitters of the perforant path (Baudry & Lynch, 1981; Di Lauro, Schmid & Meek, 1981; Storm-Mathisen, 1977; Yamamoto & Sawada, 1982). GABA is the most likely transmitter of the inhibitory basket cells (Adamec, McNaughton, Racine & Livingston, 1981; Storm-Mathisen & Fonum, 1971). Cholinergic (Mosko, et al, 1973) and catecholaminergic (Loy, et al., 1980; Srebro, Azmitia & Winson, 1982; Winson, 1980) fibers are also present in the dentate.

Because of the relatively simple and homogeneous cytoarchitecture of the dentate, intracellular events are mirrored by large, extracellular population responses (Andersen, et al., 1966; Lomo, 1971a). Stimulation of perforant path fibers produces a depolarization of granule cells, recorded intracellularly (Andersen, et al., 1966; Deadwyler, Dudek, Cotman & Lynch, 1975; Dudek, et al., 1976; Lomo, 1971a). These intracellular depolarizations, evoked nearly simultaneously by the <u>en passage</u> terminations of the pathway, are recorded as a large, negative, synchronous population EPSP in the molecular layer. The dendrites are a positive sink for depolarizing current flow. The granule cell layer and dentate hilus are a source for this current, and thus the population EPSP is recorded as a positive potential in the hilus. At high stimulation intensities, a population "spike" is superimposed on the EPSP. This population spike corresponds to synchronous granule cell discharge (Andersen, et al., 1966; Lomo,

1971a) and is recorded as a negative potential in the hilus and a positive potential in the molecular layer.

Development: As stated in the Introduction, the rat dentate gyrus undergoes major maturational changes postnatally. Eighty percent of adult granule cells are formed postnatally (Altman & Das, 1965; Schlessinger, et al., 1975). Granule cell neurogenesis begins around day 14 post-conception and continues for several months, possibly into adulthood (Altman & Das, 1965; Bayer, 1982; Bayer, Yackel & Puri, 1982; Schlessinger, et al., 1975). Neurogenesis peaks at PN5-PN8 (Schlessinger, et al., 1975; although Altman & Das (1965) suggest this peak may occur as much as a week later, at PN15). Neurogenesis in the suprapyramidal blade precedes that in the infrapyramidal blade, and the more caudally located neurons develop earlier than those located more rostrally (Schlessinger, et al., 1975).

Synapse formation begins by PN4 on those cells present (Crain, et al., 1973). Initial synaptic contacts are made on granule cell dendritic shafts and somas (Cotman, et al., 1973), with axodendritic synapses appearing prior to axosomatic (Schwartz, Pappas & Purpura, 1968; Schwartzkroin, et al., 1982). Synaptic density reaches adult levels by PN25 (Crain, et al., 1973).

The majority of synapses on mature granule cells are on dendritic spines (Nafstad, 1967), which first appear near the end of the first postnatal week (Cotman, et al., 1973). Spine density increases in a bimodal fashion, with peaks at PN14 and the adult stage (Duffy & Teyler, 1978a,b). Spine morphology increases in complexity as the animal matures past PN25 (Cotman, et al., 1973).

Perforant path fibers are present in the outer half of the molecular layer by PN4 (Loy, et al., 1977). Myelination of these fibers begins during the second or third postnatal week (Cotman, et al., 1973). Other inputs to the hippocampal formation, such as the cholinergic septohippocampal pathway, also arrive before, or shortly after birth (Crutcher, 1982; Matthews, et al., 1974; Nadler, et al., 1974).

The small size of granule cell somata makes intracellular recording difficult, even in the mature dentate, thus very little is known about the electrophysiological properties of immature granule cells. However, perforant path stimulation is capable of producing population EPSP's in the dentate by PN7 (Duffy & Teyler, 1978a, b). Furthermore, the mossy fibers are capable of discharging CA3 pyramidal cells by PN7-PN15 (Bliss, Chung & Stirling, 1974).

CHAPTER II. PAP IN THE MATURE NEOCORTEX.

Although PAP has been described in great detail in the hippocampal formation, it has not been examined to any major extent in the neocortex (Clare, et al., 1961; Racine, et al., 1975). Therefore, as a first step toward an analysis of postnatal development of neocortical PAP, the characteristics of PAP in the mature neocortex were determined. Specifically, the objective of the present experiment was 1) to determine the magnitude, duration and requirements for induction of facilitation, STP and LTP of the mature TCR, and 2) to compare PAP of the mature TCR with that reported for other forebrain pathways.

EXPERIMENT I. THE EFFECT OF SINGLE CONDITIONING PULSES

The most transient form of PAP is facilitation and is generally produced by single conditioning pulses. Experiment I was done to determine the characteristics of facilitation in the neocortex.

METHODS

Adult male hooded rats, born in our colony or obtained from Blue Spruce Farms (Altamount, N.Y.), were used in these experiments. Animals were anesthetized with intra-peritoneal injections of sodium pentobarbital (65mg/kg) and mounted in a stereotaxic apparatus. Body temperature was maintained at 34±2° C with a heat lamp.

The skull was exposed and holes drilled in homologous sites over both hemispheres of the neocortex corresponding to either Krieg's Area 8 (anterior) or Krieg's Area 2 (posterior) (Krieg, 1946). The recording electrode was either a Bakelite-coated, tungsten microelectrode (1-

2 Mohm) or a teflon-coated, stainless-steel monopolar electrode (0.28 mm diameter) and was placed in one hemisphere, 0.0-0.3 mm below the cortical surface. Ground and reference electrodes were attached to the opened scalp. Responses were amplified with a Grass P-15 pre-amplifier and a Grass EEG amplifier. Cutoff frequencies were set at 1 Hz and 3 kHz. All responses were stored and analyzed on an LSI-11 computer. Stimulation was presented via a bipolar, teflon-coated, stainless-steel electrode (0.28 mm diameter). The stimulating electrode was placed directly into the callosal fibers in the opposite hemisphere. Stimulation consisted of biphasic square-wave pulses with 0.1 msec duration each half-cycle, delivered through constant current isolation units from a Grass S-88 stimulator. Placement of the electrode into the fibers was initially verified electrophysiologically. The evoked response at the recording site showed a noticable increase in amplitude and some decrease in latency as the stimulating electrode moved from the cell layers into the callosum. In addition, in some animals electrode placements were verified histologically.

The effects of a single conditioning pulse on the amplitude of subsequent test pulses (Paired-Pulse) was examined for both the anterior TCR (aTCR) and the posterior TCR (pTCR). Pulse pairs were delivered once every 10 to 15 seconds with inter-pulse intervals (IPI's) ranging from 20 - 5000 msecs. Five pairs were delivered at each interval. Conditioning and test stimuli were of equal intensity. Stimulus intensity was set at 50-75% of maximum on a stimulus/response (I/O) curve. This I/O curve was determined by varying stimulus intensity from 10 uA-1200 uA and recording response amplitude. The 50-75% range was

chosen to avoid any ceiling effects on facilitation (Racine & Milgram, 1983). Response amplitude was measured by calculating the slope (mV/msec) of the falling positivity-rising negativity. This measure correlated well with peak to peak amplitude (Pearson $r > 0.95$). Percent paired-pulse facilitation/depression was calculated as test response amplitude divided by conditioning response amplitude. Time constants of decay for facilitation were determined from a least-squares linear regression of the log of post-conditioning response amplitude.

RESULTS

BASELINE RESPONSE CHARACTERISTICS. Single pulses applied to the corpus callosum produced a biphasic, positive-negative, or monophasic, negative, trans-callosal potential (TCR) recorded at the surface of the opposite hemisphere of the anterior and posterior neocortex (Figure 1). The biphasic potential (as described by Chang, 1953; Clare, et al., 1961; Curtis, 1940a,b; Grafstein, 1959) was seen most often in the anterior neocortex. The amplitude of the aTCR (maximal aTCR amplitude, averaged across animals, = 2096 uA) was significantly larger than the pTCR (maximal pTCR amplitude, averaged across animals, = 418 uA) ($t = 4.31$, $df = 13$, $p < 0.01$).

PAIRED-PULSE EFFECTS. Single conditioning pulses resulted in a significant increase in the amplitude of responses to subsequent test pulses at short IPI's, in pentobarbital anesthetized mature animals (Figure 2). The magnitude of facilitation peaked for both the aTCR and pTCR between 20 - 100 msec IPI. The magnitude of peak facilitation, however, differed markedly between the two responses. Whereas paired-pulse facilitation of the aTCR peaked at a mean of 114%, pTCR

Figure 1. Representative examples of anterior (A) and posterior (B) transcallosal responses, before and after PAP. Stimulus intensity was set at approximately twice threshold level.

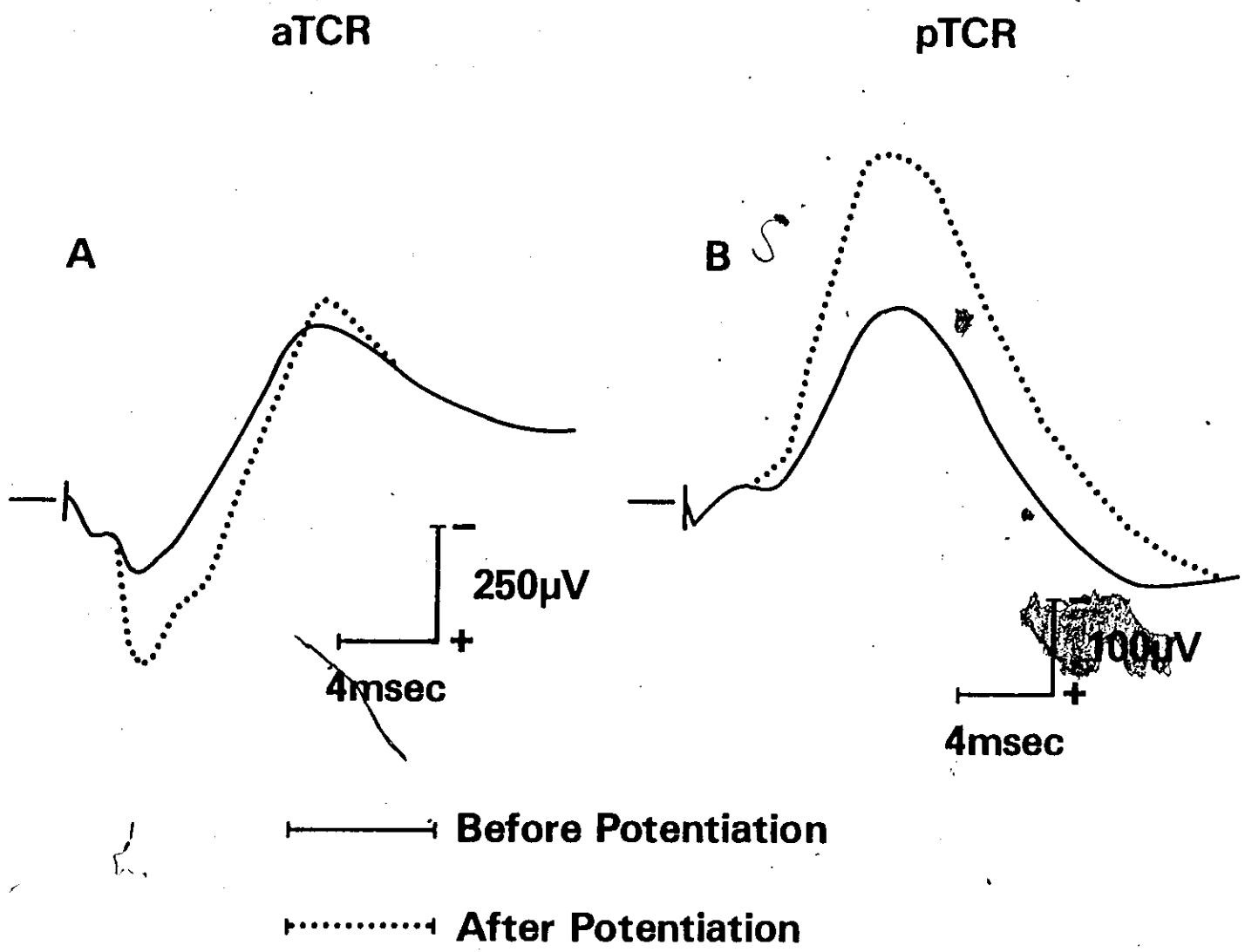
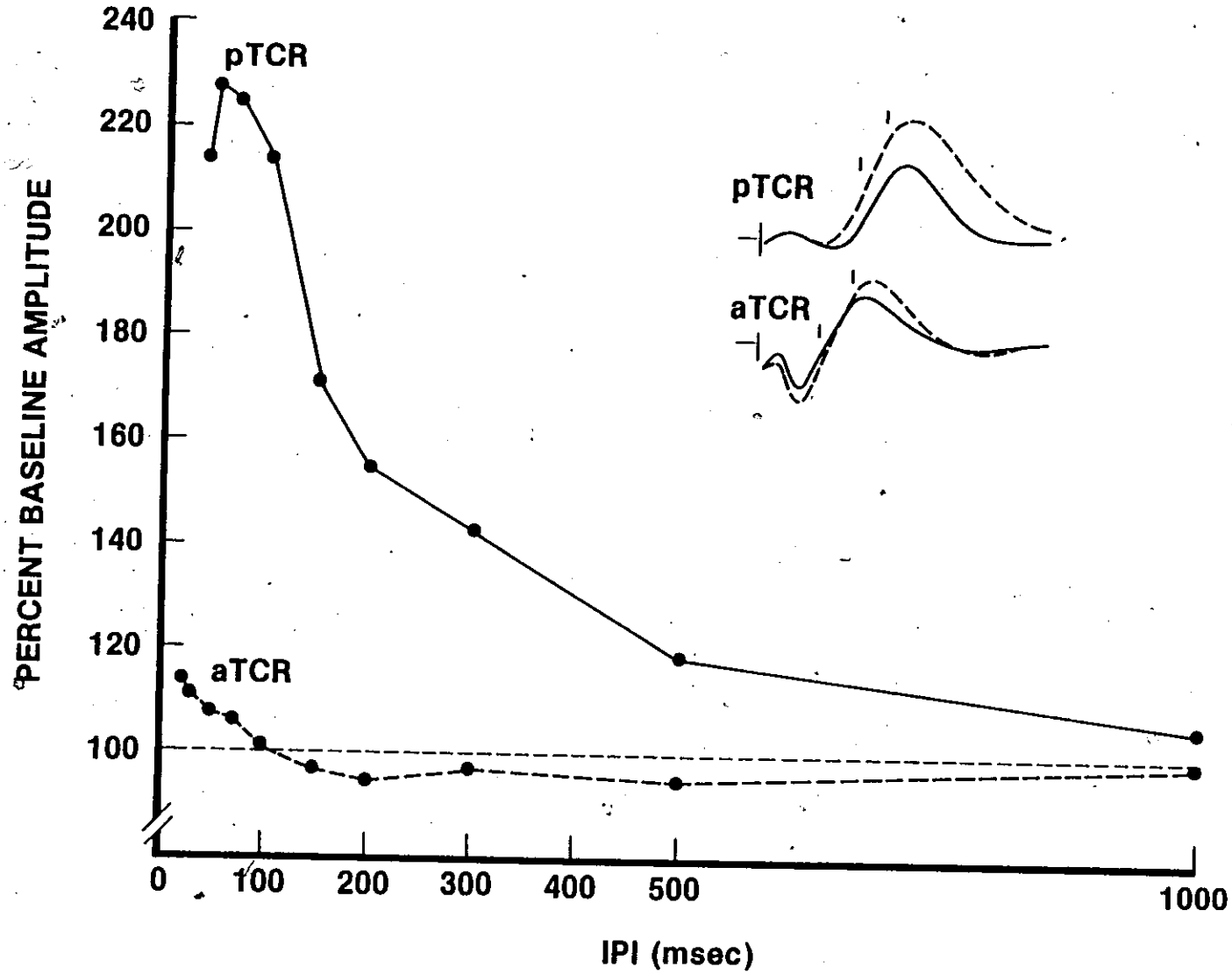

aTCR
pTCR
A
B
250μV
4msec
100μV
4msec
Before Potentiation
After Potentiation

Figure 2. Test response amplitude of the aTCR and pTCR following single test pulses, expressed as a percentage of baseline response amplitude and inter-pulse interval (IPI). Evoked responses are representative baseline (solid) and test (dashed) responses at the peak of facilitation. Responses amplitude was measured as the slope between the cursors above each response.


240
220
200
180
160
140
120
100
PERCENT BASELINE AMPLITUDE
pTCR
aTCR
pTCR
aTCR
0
100
200
300
400
500
1000
IPI (msec)

facilitation reached a mean peak of 228%. This was a statistically significant difference (Mann-Whitney U, $p < 0.01$). Facilitation decayed approximately exponentially for both the aTCR and pTCR, with time constants of 33 msec and 306 msec, respectively.

In some cases, maximal facilitation appeared to be masked by an initial depression at very short IPI's, suggesting a possible inhibitory component to the paired-pulse effects (Andersen, et al., 1966; Szentagothai, 1969) or a transient depletion of transmitter substance (McNaughton, 1981).

EXPERIMENT II. THE EFFECT OF HIGH FREQUENCY STIMULATION

The longer-lasting forms of PAP, STP and LTP, are produced by application of conditioning trains of pulses. Experiment II examined STP and LTP of the aTCR and pTCR.

METHODS

The effects of high frequency stimulation on the TCR were determined in anesthetized adult animals. Animals were prepared as above. Test pulse intensity was approximately 50-75% of maximum. Single test pulses were applied at 0.1 Hz for 10 to 20 minutes to determine baseline variability. Following baseline, a single train (400 Hz, 100 msec) was delivered, followed by an additional 20 minutes of test pulses. A total of 2-6 trains with subsequent test pulses, were applied consecutively in this manner. The majority of pTCR animals were run prior to aTCR animals. From the pTCR data, it was determined that post-train response amplitude generally reached a stable baseline within 10 minutes (see Figure 4). Therefore subsequent testing, including most

of the aTCR animals, utilized a 10 minute inter-train interval. Varying inter-train interval between 10 and 20 minutes in several pilot animals had no effect on STP or LTP magnitude or decay rates.

Train intensity was varied from 100-1200 uA, depending on the area stimulated. Results from pilot animals suggested that posterior placements were more susceptible to stimulation-induced after-discharges (AD's) than anterior placements, thus lower train intensities were used with posterior placements. Post-train response amplitude was expressed as a percentage of baseline amplitude. The magnitude of STP was defined as the maximal percent change, 10-60 seconds post-train. The magnitude of LTP was defined as the percent increase above baseline at least 10 minutes post-train. All determinations of STP and LTP magnitude were done following the first train of a series. Time constants of decay for STP were determined from a least-squares linear regression of the log of post-train response amplitude.

RESULTS

High frequency stimulation of callosal fibers produced a marked, long-lasting increase in the subsequent amplitude of both the aTCR and pTCR. STP was rarely seen in the absence of LTP (discussed below). Even though lower-train intensities were used with the pTCR than the aTCR, to avoid AD's, the magnitude of STP and LTP of the pTCR was significantly larger than that of the aTCR (Mann-Whitney U, $p < 0.01$ for both STP and LTP). (See Figures 3 and 4). The mean peak magnitude of STP of the aTCR was 134 ± 27% (range 110-178%), and of the pTCR, 305 ± 93% (range 200-486%). Mean LTP magnitude was 107 ± 6% (range 100-115%) and 166 ± 43% (range 108-250%) for the aTCR and pTCR, respectively.

Figure 3. A. Representative aTCR preceding (solid) and following (dashed) high frequency stimulation. Calibration is 500uV, negative up, and 2 msec. B. aTCR amplitude preceding and following high frequency stimulation of the corpus callosum, expressed as a percentage of baseline amplitude. High frequency conditioning trains were applied at the arrows.

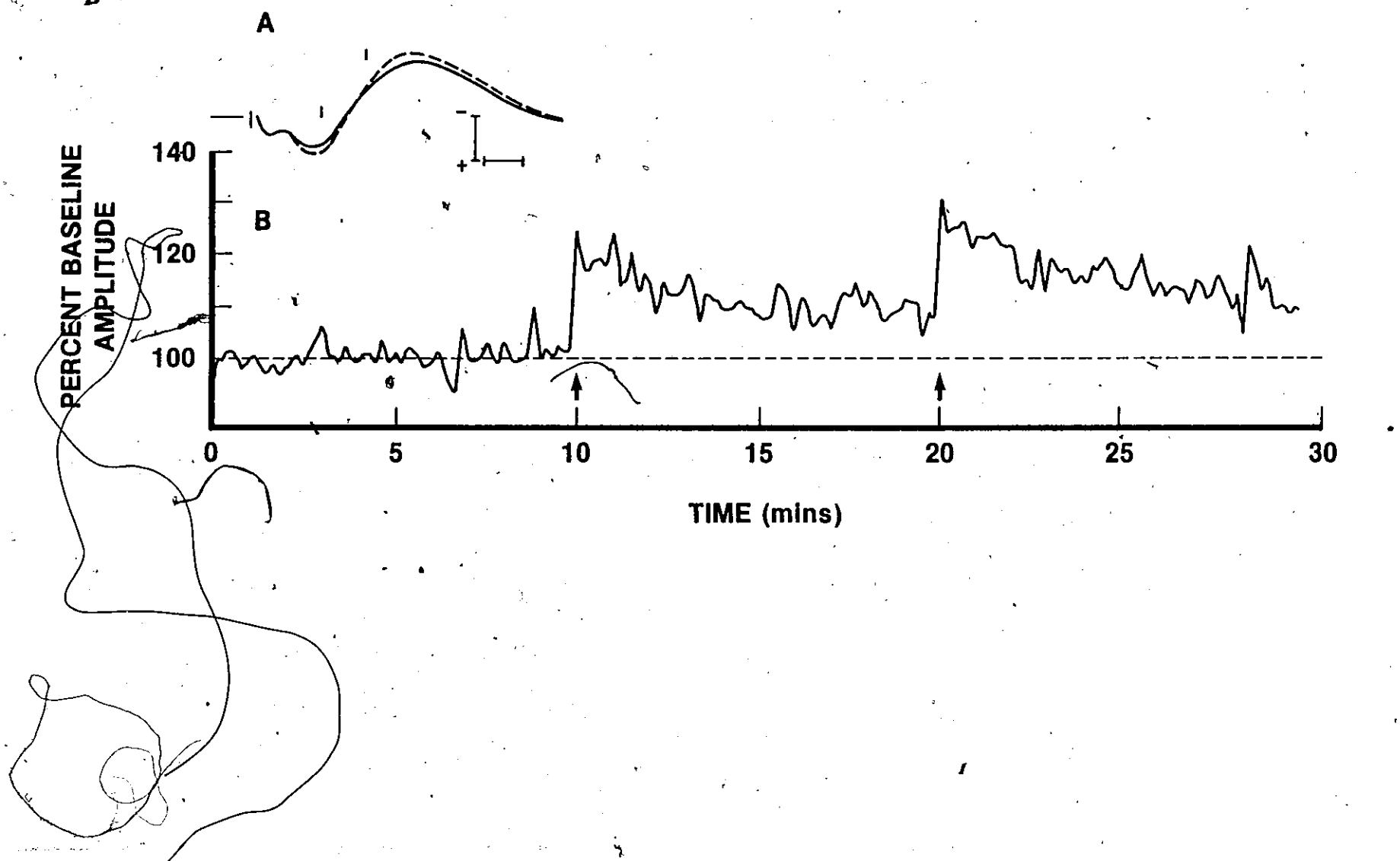
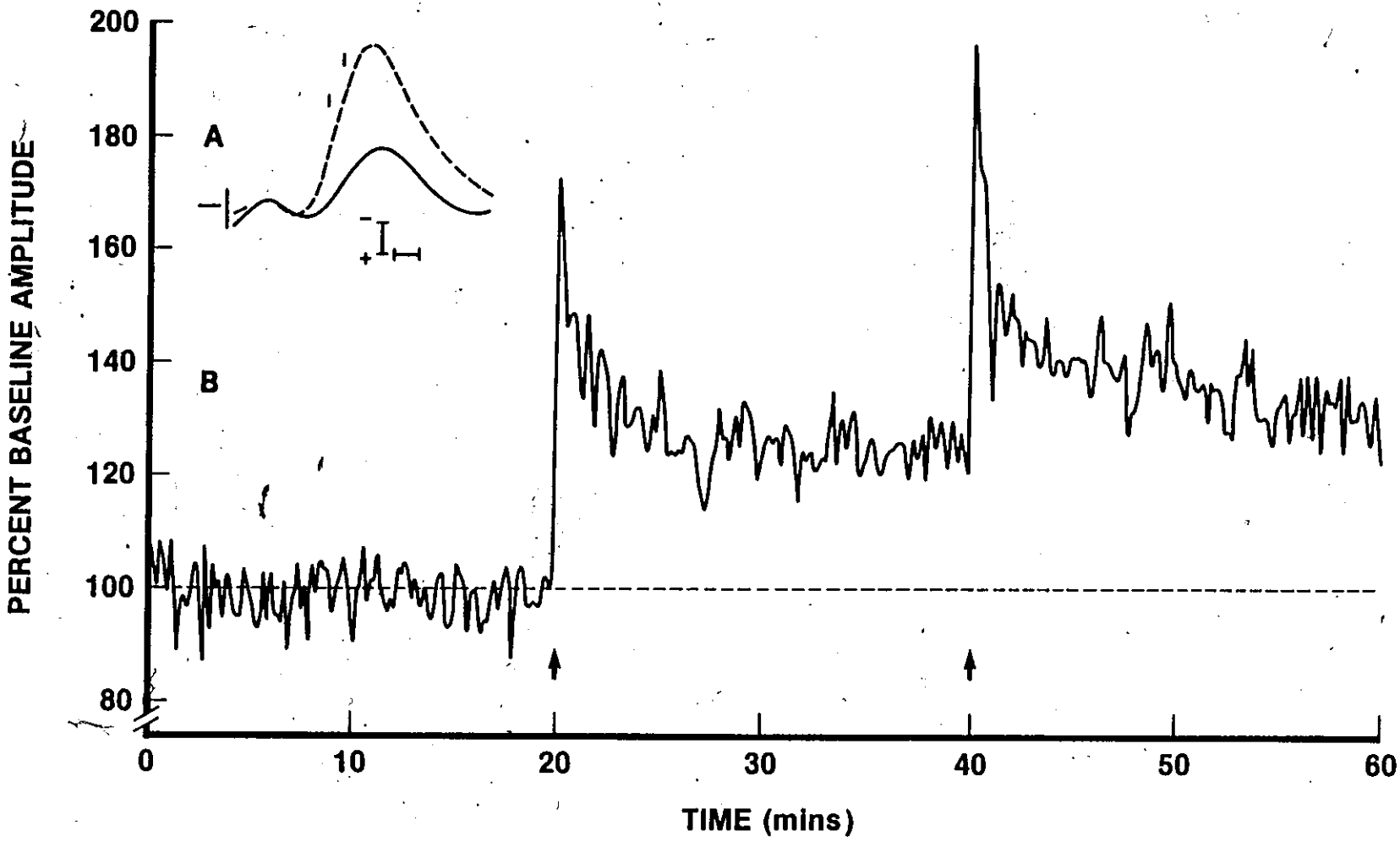

A
B
140
120
100
PERCENT BASELINE AMPLITUDE
0
5
10
15
20
25
30
TIME (mins)

Figure 4. A. Representative pTCR preceding (solid) and following (dashed) high frequency stimulation. Calibration is 250uV, negative up, and 2msec. B. pTCR amplitude preceding and following high frequency stimulation of the corpus callosum, expressed as a percentage of baseline amplitude. High frequency stimulation trains were applied at the arrows.


PERCENT BASELINE AMPLITUDE
200
180
160
140
120
100
80
A
B
I
0
10
20
30
40
50
60
TIME (mins)

The decay of PAP in both placements could be described exponentially. PAP decay of the pTCR could generally be described by two exponential components, with time constants of 72-105 sec and 397-936 sec. In some cases, the short lasting component was not present. PAP decay of the aTCR was well described by a single exponential, with a time constant of 330-587 sec.

DISCUSSION

The results of the present experiment demonstrate that PAP can be induced in the mature neocortex, and that the characteristics of neocortical PAP are similar to those reported for other forebrain pathways (Bliss & Lomo, 1973; McNaughton, 1980; Racine & Milgram, 1983; Racine, et al., 1983). Both the aTCR and pTCR demonstrate facilitation, STP and LTP. The time constants of decay of facilitation and STP correspond well with those reported at the n.m.j. (Magleby & Zengel, 1976a) and limbic forebrain pathways (Lomo, 1971; McNaughton, 1980; McNaughton, 1982; Racine & Milgram, 1983). Furthermore, these results suggest that the magnitude of neocortical evoked potentials and PAP vary across functional regions within the neocortex. The anterior placements used here are in an area with relatively dense callosal terminations, while the posterior placements are in relatively light termination zones (Jacobson, 1970). Vogt & Gorman (1982) report a similar relationship between termination density and intracellular EPSP amplitude in the anterior and posterior cingulate cortex, in vitro. Stimulation of the corpus callosum produces small EPSP's in the posterior cingulate cortex where callosal terminations are sparse compared to the anterior cingulate where terminations are more dense and EPSP's are larger.

Although this would account for the difference in response amplitudes, an explanation of the inverse relationship between response amplitude and PAP magnitude seen with the aTCR and pTCR requires further investigation.

One possible explanation for greater PAP of the pTCR is that stimulation of the corpus callosum in this region also involves stimulation of the dorsal hippocampal commissure. The commissural input to the hippocampus has been reported to demonstrate PAP (Buzsaki, 1980). Perhaps then, the potentiation of hippocampal potentials increases the amplitude of surface-recorded cortical potentials recorded above the hippocampus, through volume conduction. This explanation is unlikely for the following reasons: 1) the pTCR is recorded as a surface-negative potential, suggesting the current sink, and thus the activated synapses are near the tip of the recording electrode, i.e. in the neocortex (Hubbard, Llinas & Quastel, 1969; Rall & Shepard, 1968). 2) Depth profile analysis of the pTCR shows the pTCR reverses in polarity approximately 1-1.5 mm below the cortical surface (personal observation). This again suggests that the surface-negative potential is due to a current sink, and activated synapses, in the neocortex, and is not a current source for potentials generated in the hippocampus.

CHAPTER III. POSTNATAL DEVELOPMENT OF PAP IN THE NEOCORTEX

The experiments in Chapter II demonstrated that mature neocortical synapses are capable of supporting short-term and long-term potentiation. However, many anatomical and physiological aspects of the mature neocortex do not develop until several weeks postnatally in the rat (see Intro.). The experiments in this chapter were designed to describe the development of PAP in the neocortex. In addition, correlation of PAP development to known structural and neurochemical developments in the neocortex is discussed.

The postnatal development of PAP in the neocortex was examined using the aTCR. Although the mature pTCR demonstrated a greater magnitude of potentiation than the aTCR, the aTCR was chosen for two major reasons, 1) the mature aTCR had a lower threshold and larger amplitude than the pTCR, and 2) the aTCR did not appear to be as susceptible to AD's as the pTCR.

EXPERIMENT I. THE EFFECT OF SINGLE CONDITIONING PULSES

The following experiment was designed to describe the postnatal development of paired-pulse facilitation/depression of the aTCR.

METHODS

Male hooded rats were tested once, between PN7 and adult (>PN180). Date of birth was PN0. Animals were anesthetized with intraperitoneal injections of sodium pentobarbital. Due to the increased sensitivity to barbiturates in young animals (Bianchine & Ferguson, 1967; Mirkin, 1970), pentobarbital dosage was varied to provide similar levels of anesthesia in all age groups. Dosage was 20 mg/kg for animals

less than 20-30 g, and 65 mg/kg for animals over 20-30 g. These dosages provided surgical levels of anesthesia for 1-2 hours.

Due to the small size and delicate nature of the neonate skull, a special stereotaxic adaptor was used for some animals less than 2 weeks old. This adaptor consisted of a small tray filled with clay or dental acrylic molded to the shape of the neonate's body. The clay or acrylic was molded so that the dorsal skull surface was maintained in a horizontal position. The tray was suspended between the ear bars and clamped into the incisor bar on the stereotaxic frame. The electrodes were placed using standard stereotaxic electrode carriers. The electrodes and placements were the same as those described previously for adults. Medial-lateral placement of the stimulation and recording electrodes was held constant across age groups, as this dimension changes only slightly after the first postnatal week (Valenstein, Case & Valenstein, 1969). In some cases electrode placements were verified histologically. Body temperature was maintained at 34±2C with a heat lamp during testing.

Paired-pulse facilitation/depression of the immature TCR was examined as described for mature animals in Experiment I. However, due to the long latency and duration of immature responses, paired-pulse effects could not be accurately described for IPI's of less than 40 msec in some age groups. Data were analysed as described above.

RESULTS

BASELINE RESPONSE CHARACTERISTICS. Single pulses applied to the corpus callosum produced a biphasic, positive-negative aTCR in all age groups. The response underwent a decrease in threshold, latency and halfwidth,

and an increase in amplitude with age. Mean values of these measures for each age group are given in Table I.

PAIRED-PULSE EFFECTS. The effect of a single conditioning pulse on subsequent test responses in the immature neocortex is displayed in Figure 5. Results from the mature aTCR are reproduced for comparison. Single conditioning pulses produced a large (up to 50%) and long-lasting (500-3000 msec) depression in immature animals as compared to the brief facilitation seen in adults. The magnitude and duration of this depression varied in an age dependent manner, being most profound in younger animals (e.g. 500 msec IPI, Kruskal-Wallace, $\chi^2=125.9$, df=3, $p < 0.001$).

EXPERIMENT II. THE EFFECT OF HIGH FREQUENCY STIMULATION

The results of Experiment I suggest that the effects of single conditioning pulses varies with age. The present experiment was an examination of the development of STP and LTP of the aTCR.

METHODS

Test pulses producing 50-80% maximal responses were applied to the corpus callosum at 0.1 Hz for 10 minutes preceding and following a single, high intensity train (1200 uA, 400 Hz, 100 msec). All the results presented here are from animals undergoing this standard procedure. In addition, some animals received one or more trains of varied frequency (10-400 Hz) and duration (50 msec - 10 seconds). Results from these additional animals did not appear to differ from those of animals receiving the standard train, and thus are not included

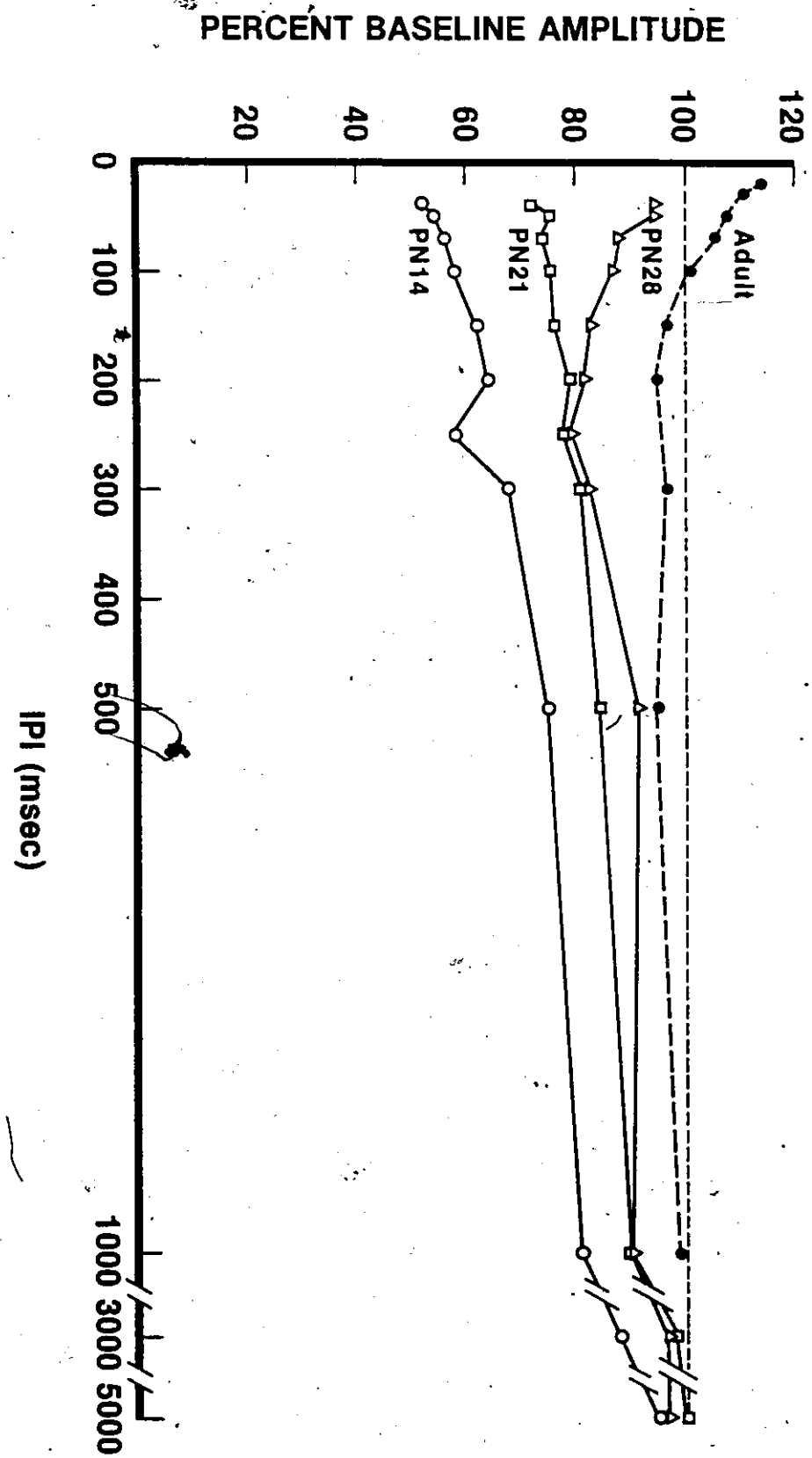
TABLE I. Quantitative development of the surface-recorded aTCR in anesthetized rats.

Table I

Quantitative development of the surface-recorded aTCR in anesthetized rats (mean ± S.D.). Latency to response onset, halfwidth (measured from baseline to the peak of the negative component), threshold, and maximal peak to peak response best characterized post-natal response development.

Postnatal Age (days)	Body Weight (grams)	Latency to initial positivity (msec)	Response half-width Baseline to peak negativity (msec)	Response threshold (μA)	Maximal response to 1000μ stimulation (μV)
7	16.3 ± 2.2	13.5 ± 2.2	21.7 ± 0.3	200	217 ± 83
14	30.7 ± 6.1	11.6 ± 1.5	18.4 ± 2.4	127	1450 ± 507
21	44.2 ± 5.3	6.5 ± 1.3	10.5 ± 1.6	70	1497 ± 843
28	78.3 ± 9.7	3.7 ± 0.7	9.4 ± 2.1	72	828 ± 259
35	111.8 ± 25.2	2.9 ± 0.6	8.8 ± 1.3	38	1014 ± 310
Adult	332.8 ± 88.2	2.2 ± 0.3	6.7 ± 1.0	30	1190 ± 508

Figure 5. The effect of single conditioning pulses on aTCR amplitude across ages, expressed as a percentage of baseline amplitude and inter-pulse interval. Note that only adult animals showed any net facilitation.


PERCENT BASELINE AMPLITUDE
120
100
80
60
40
20
0
Adult
PN28
PN21
PN14
100
200
300
400
500
1000
3000
5000
IPI (msec)

here. Data were analysed as previously described for the mature animals.

RESULTS

The incidence of STP/LTP increased with age, as shown in Figure 6. STP could not be consistently produced until after PN16. Prior to this age, most animals showed no post-train effect at all. In those instances where a post-train potentiation was apparent, it was generally of small magnitude and decayed very rapidly (see Figure 7), suggesting a possible facilitation or augmentation effect rather than potentiation (as defined by Magleby & Zengel, 1976 and McNaughton, 1982).

LTP appeared several days after STP, between PN18 and PN21. Once detected, the magnitude of both STP and LTP approached adult levels rapidly. Representative, averaged potentiation runs for several age groups are shown in Figure 7, along with examples of TCR's from each age group. STP decay time constants calculated (where possible) for immature animals were within the range for adult animals. The development of STP and LTP in the neocortex is summarized in Table II.

DISCUSSION

The results of the present experiments suggest that PAP in the rat anterior neocortex develops over a short period during the first few postnatal weeks. STP develops several days prior to LTP. Once present, the magnitude of both STP and LTP rapidly approach adult levels. Paired-pulse facilitation, however, did not appear until after PN35. This is surprising in light of the similarity between the mechanisms of facilitation and STP. It is possible that facilitation is present in immature animals, but masked by a greater susceptibility to transmitter

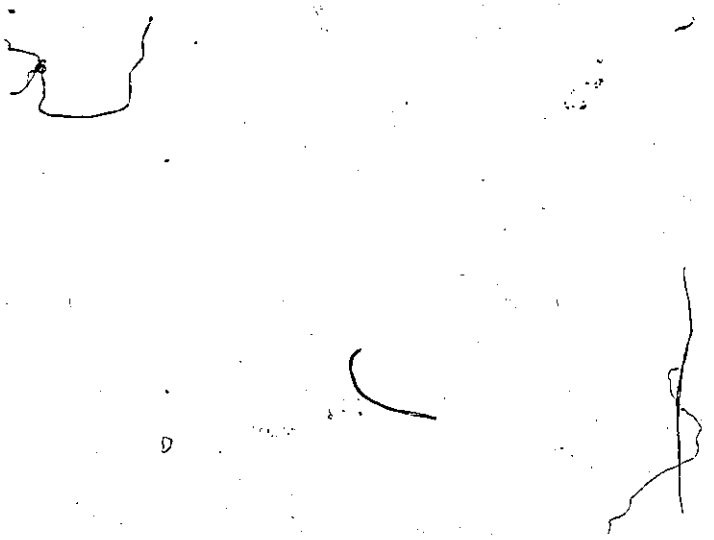
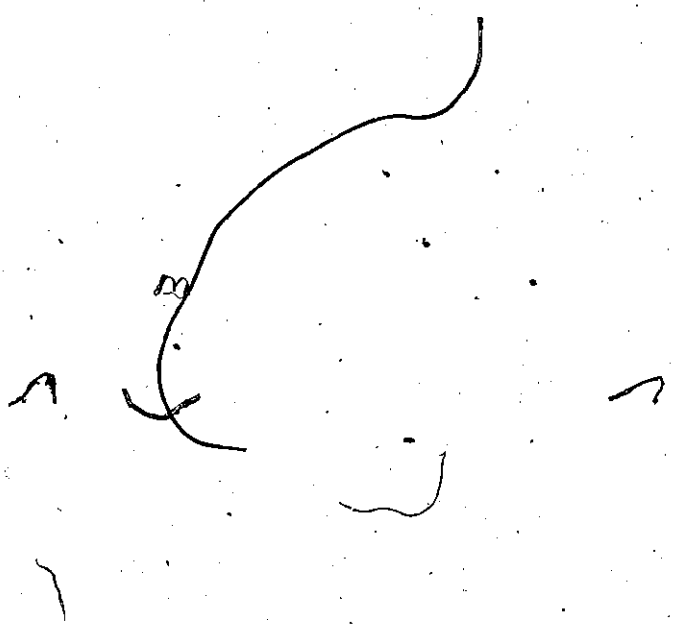


Figure 6. Percent of animals per age group demonstrating STP (solid line) and LTP (dashed line) of the aTCR, following high frequency stimulation.



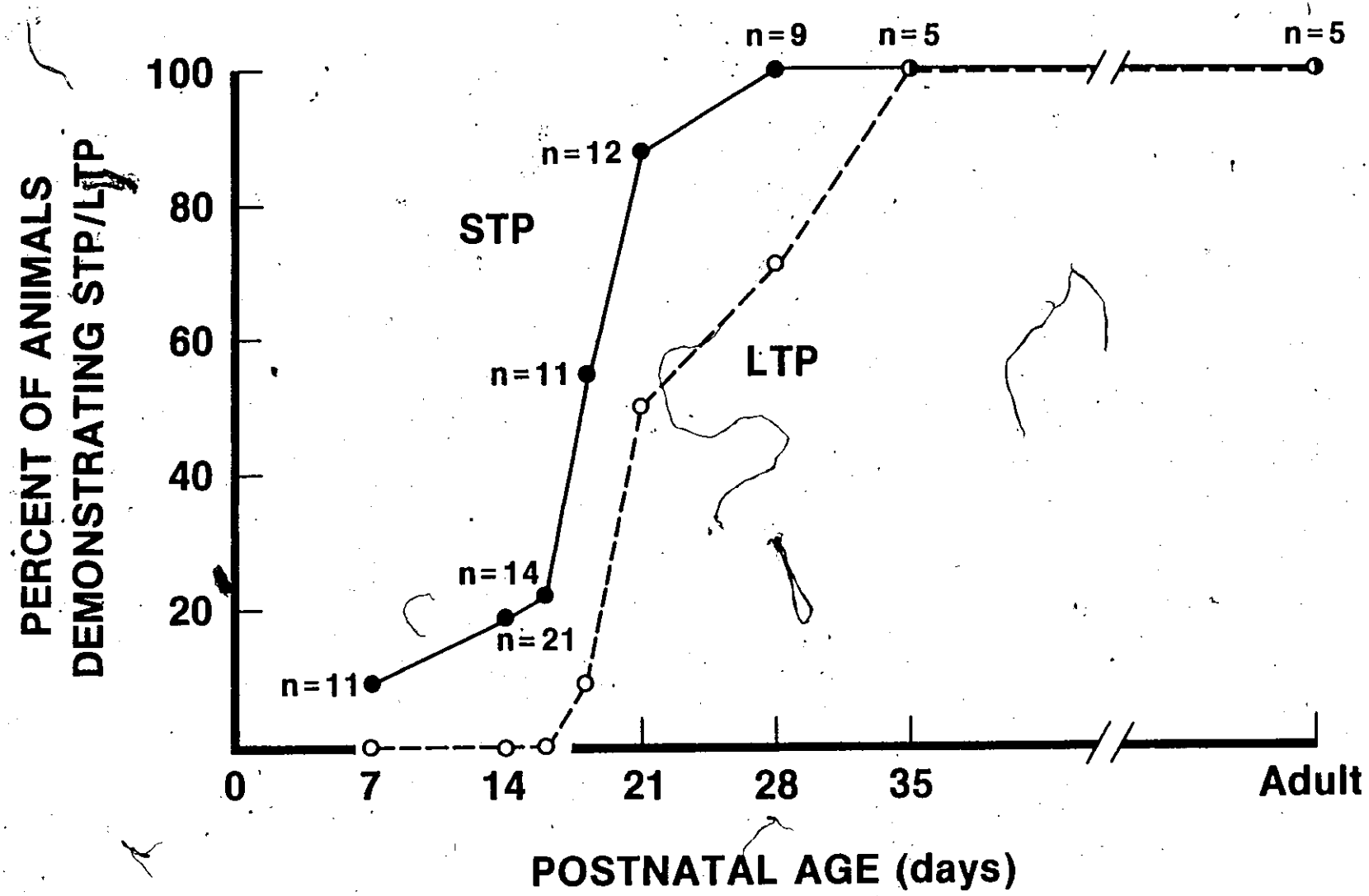

PERCENT OF ANIMALS DEMONSTRATING STP/LTP
100
80
60
40
20
0
7
14
21
28
35
Adult
POSTNATAL AGE (days)
STP
LTP
n=11
n=14
n=21
n=11
n=12
n=9
n=5
n=5

Figure 7. Amplitude of aTGR expressed as a percent of baseline amplitude, preceding and following identical high frequency trains in several age groups. On the right are representative responses for each age group. Calibration is 500uV and 5 msec.

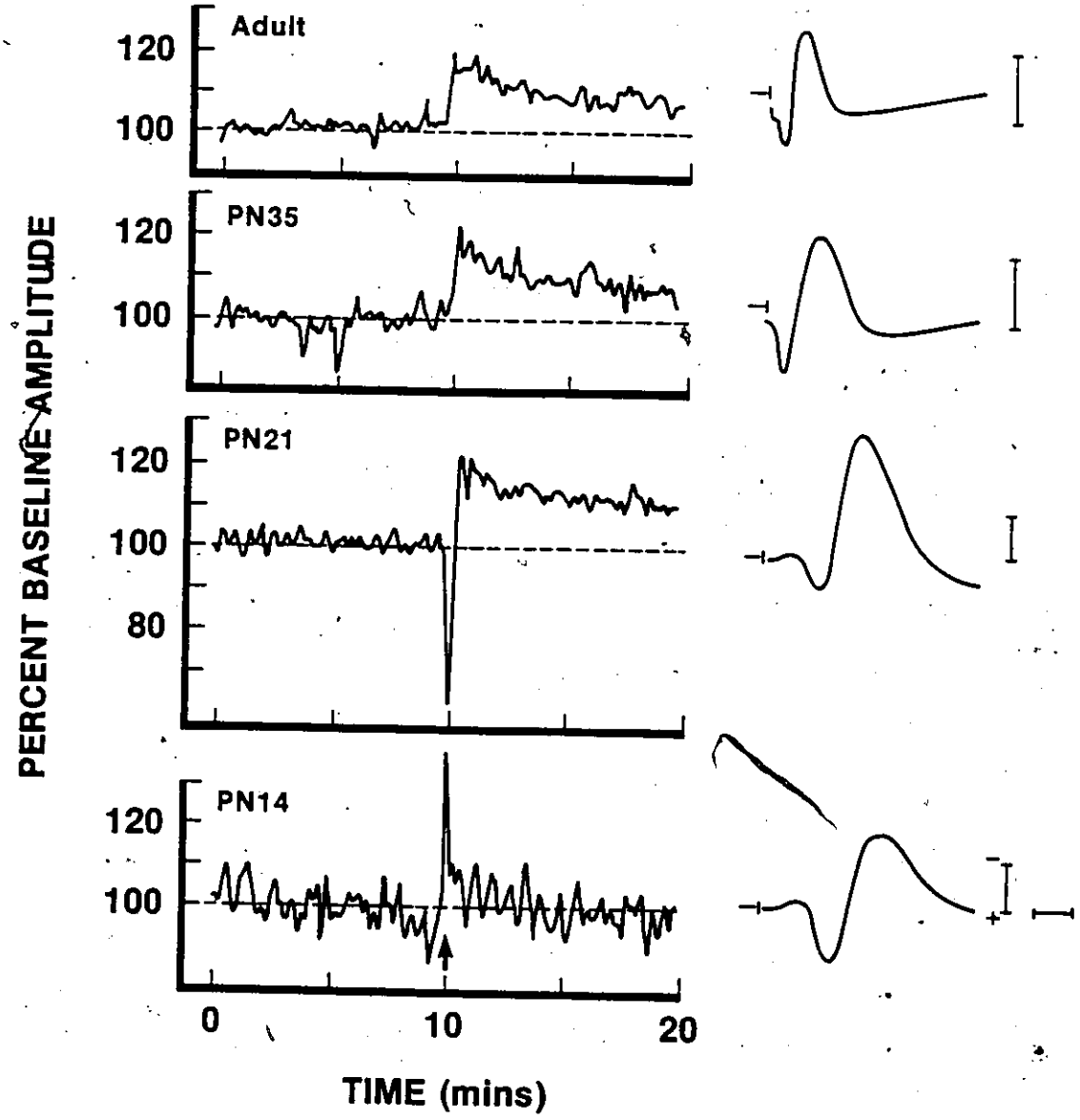

Adult
120
100
PN35
120
100
PN21
120
100
80
PN14
120
100
0
10
20
PERCENT BASELINE AMPLITUDE
TIME (mins)

TABLE II. Percent demonstrating, and magnitude of, STP and LTP in the neocortex, by age group.

TABLE II

Percent demonstrating, and magnitude of, STP and LTP in each age group, in the neocortex. STP and LTP magnitudes are expressed as percent of baseline, 10 seconds and 10 minutes post-train, respectively (median ± inter-quartile range). Weight (g) is mean ± S.D.

Age (days)	N	Weight (g)	% Demon. STP	Magnitude STP	% Demon. LTP	Magnitude LTP
7	11	16.3 ±2.2	9	100 ±0.0	0	100 ±0.0
14	21	30.7 6.1	19	100 0.0	0	100 0.0
16	14	32.5 3.6	22	100 0.0	0	100 0.0
18	11	40.2 5.4	55	123 6.0	9	100 0.0
21	12	44.2 5.3	88	125 10.5	50	102 5.0
28	9	78.3 9.7	100	115 5.0	71	105 1.5
35	5	111.8 25.2	100	120 3.0	100	110 1.0
Adult	5	332.8 88.2	100	130 11.0	100	106 2.5

depletion or greater recurrent or feed-forward inhibition in neonates. This problem will be examined in greater detail in subsequent experiments.

No strong correspondence was noted between neocortical STP/LTP development, reported here, and known structural developments, such as synaptogenesis (Aghajanian & Bloom, 1967), myelinogenesis (Seggie & Berry, 1972) and dendritic spine formation (Wise, et al., 1979). Furthermore, during the period of rapid PAP development, TCR morphology does not undergo any corresponding sudden changes (e.g. threshold or amplitude) that might be expected during a concomitant rapid structural development. These results suggest, therefore, that neocortical PAP development may be dependent on neurochemical maturation (e.g. Ca^{++} binding or protein phosphorylation) rather than on specific structural developments.

CHAPTER IV. POSTNATAL DEVELOPMENT OF PAP IN THE DENTATE GYRUS

Although dentate granule cell neurogenesis continues well into the postnatal period in the rat (Altman & Das, 1965; Schlessinger, et al., 1975), development of cellular structure and connections in the dentate precedes that in the neocortex (see Intro.). Using the same procedures as in the previous chapter, the present experiments were an attempt to describe the postnatal development of PAP in the dentate gyrus. The difference in the rate of structural and neurochemical development between the two systems should produce a difference in the age of PAP development.

PAP of the perforant path-granule cell synapse has been well described in mature animals (Bliss & Lomo, 1973; Douglas & Goddard, 1975). Nevertheless, in order to facilitate comparison, adult animals were included in the present study.

EXPERIMENT I. THE EFFECT OF SINGLE CONDITIONING PULSES.

Single conditioning pulses in the mature dentate produce a brief depression followed by a pronounced facilitation (Andersen, et al., 1966; Lomo, 1971b; Racine & Milgram, 1983). The present experiment was an examination of paired-pulse effects in the immature dentate gyrus.

METHODS

Male hooded rats, aged PN7-adult were anesthetized with I.P. injections of pentobarbital, and placed in the stereotaxic as described above. Stimulation and recording electrodes were lowered into the perforant path and dentate hilus, respectively. The coordinates used

for these structures in each age group were verfied histologically and are listed in Table III. The electrodes were lowered to produce a maximal positive population EPSP and a superimposed, negative population spike. In some cases, data from younger animals (PN7-PN10) included dentate molecular layer recordings. Attempts to position the recording electrode in the hilus of these very young animals were not always successful. Although the components of responses recorded in the molecular layer are reversed in polarity, as discussed in the Introduction, they demonstrate all the characteristics of hilus potentials (e.g. PAP; Wilson, 1981; Wilson, et al., 1981; personal observation). The same criteria were used for determining the validity of responses in all cases: 1) low threshold, 2) threshold for putative EPSP lower than that for population spike, and 3) response latency appropriate for age (based on pilot animals). Results obtained with molecular layer recording appeared to correlate well with dentate hilar recording.

Although EPSP's without population spikes could be evoked at low stimulus intensities, they were generally small and highly variable. Paired-pulse effects using low intensity stimuli in pilot animals were equally variable. By far the most profound paired-pulse effects were seen in the population spike when using higher intensity stimulation. Therefore, paired-pulse facilitation/depression was examined in detail here using conditioning pulses supra-threshold for population spike, 75-100% of maximum. Paired-pulse depression is often taken as a measure of recurrent inhibition (Adamec, McNaughton, Racine & Livingston, 1981, Andersen, et al., 1966). Pulse pairs (20-5000 msec IPI) were applied to

TABLE III. Stereotaxic coordinates used for perforant path and dentate hilus placements in each group.

TABLE III

Stereotaxic coordinates used for perforant path and dentate hilus placements in each age group, lateral to midline and posterior to Bregma.

Age (days)	Incisor	Perforant Path		Dentate Hilus	
		lat	post	lat	post
7	-2.0	3.5	4.5	1.5	2.0
10	-2.0	4.0	5.4	1.5	2.0
14	0.0	4.1	5.4	1.5	2.0
21	0.0	4.3	5.4	1.8	2.0
28	0.0	4.5	5.5	2.5	3.0
35	0.0	4.5	7.0	2.5	3.0
Adult	-3.0	4.8	8.0	2.5	3.4

the perforant path, one pair/10 seconds, and responses recorded in the hilus as stated above. Five responses were recorded and averaged at each IPI.

EPSP amplitude was measured as the slope (mV/msec) of the initial rising phase of the EPSP, prior to spike onset. The population spike was measured between a line joining spike onset and offset, and spike peak.

RESULTS

BASELINE RESPONSE CHARACTERISTICS. Stimulation of the perforant path produced a positive EPSP recorded in the dentate hilus in all age groups. At higher stimulus intensities, a negative population spike was superimposed on the EPSP. Both components demonstrated decreases in threshold and latency, and an increase in amplitude with age (see Table IV).

PAIRED-PULSE EFFECTS. The results of the paired-pulse tests are summarized in Figures 8 and 9. Figure 8 displays the amplitude (slope) of the test EPSP as a percent of the conditioning EPSP. In all age groups, the test EPSP was depressed for up to 1 second. The magnitude of this depression varied with age, with younger animals showing significantly greater depression (e.g. 500 msec IPI, Kruskal-Wallace, $\chi^2=15.88$, df=3, $p < 0.005$). These results are similar to those found for the TCR (see Chapter III).

Paired-pulse effects on the population spike are displayed in Figure 9. In adults, the effects of a single conditioning pulse can be roughly described by two components: recurrent inhibition and facilitation (Adamec, McNaughton, Racine & Livingston, 1981; Andersen,

TABLE IV. Quantitative development of the perforant path-dentate hilus response in anesthetized rats.

TABLE IV

Quantitative development of the perforant path-dentate hilus response in anesthetized rats (mean ± S.D.).

Postnatal Age (days)	Body Weight (grams)	EPSP Latency (msec)	EPSP Threshold (uA)	Population Spike (uA)	Maximal Population Spike to 1000 µA stimulation
7	17.3±2.2	11.4±3.8	175±90	388±223	738±838
14	31.8±5.2	5.5±2.3	121±64	296±142	2079±1129
21	45.1±9.1	3.5±1.3	64±23	218±141	5418±3375
28	71.3±17.3	2.9±0.7	63±40	220±126	8439±5346
35	119.9±15.1	2.3±0.4	56±33	254±174	6369±7711
Adult	357±74.5	2.0±0.2	65±24	200±67	4924±5526

Figure 8. The effect of single conditioning pulses on population EPSP amplitude across ages, expressed as a percentage of baseline amplitude and inter-pulse interval.

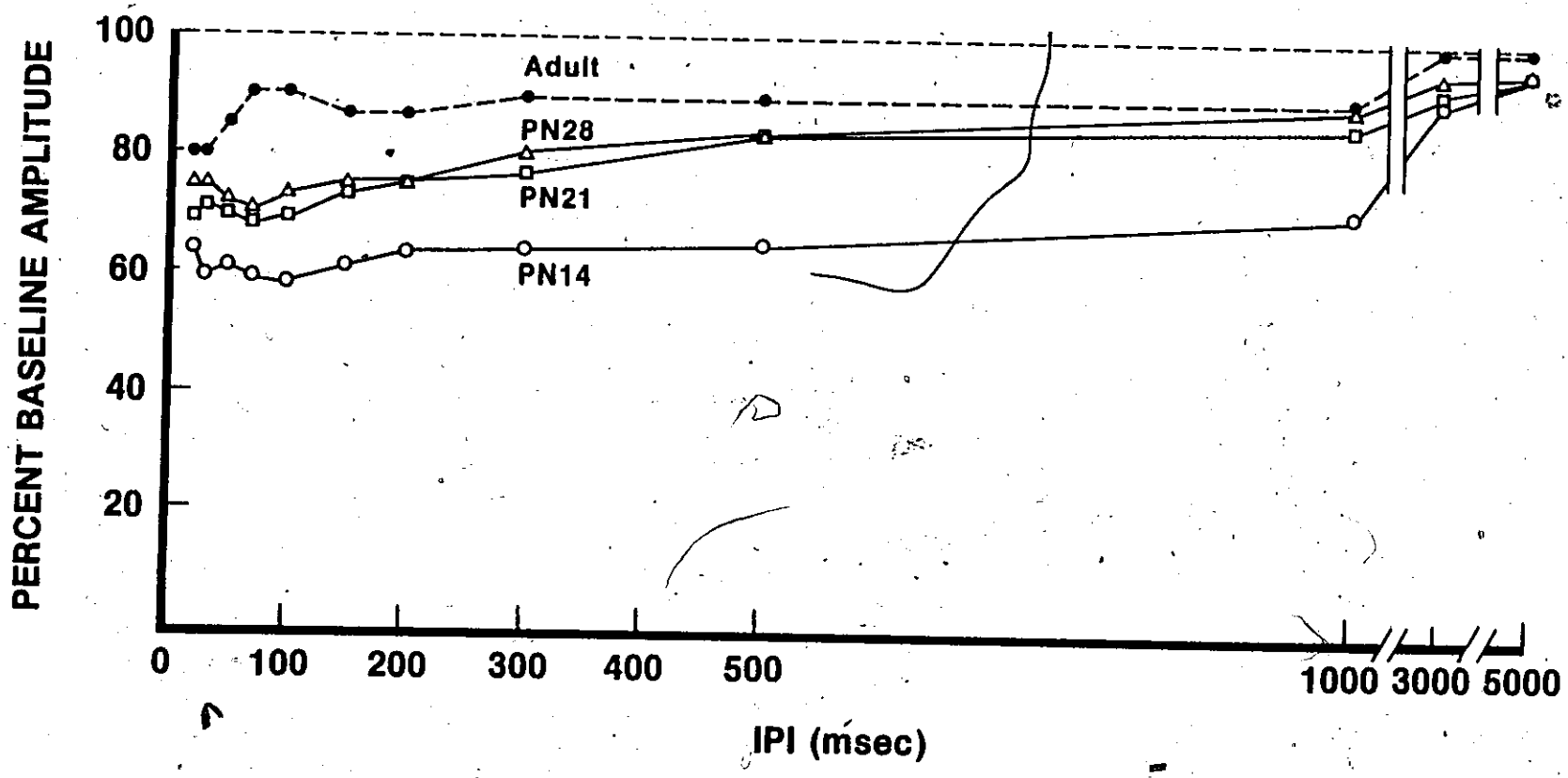
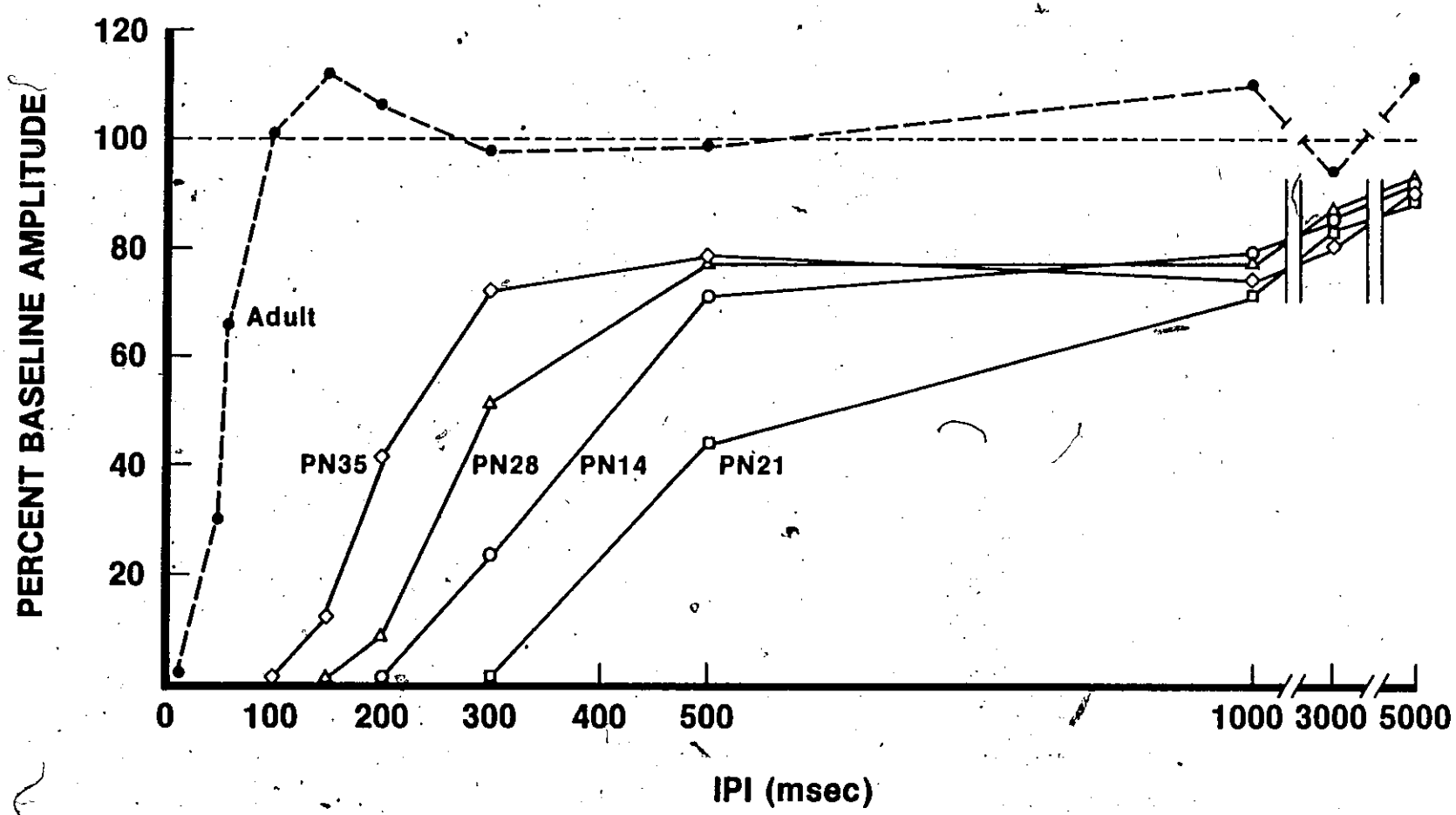

PERCENT BASELINE AMPLITUDE
100
80
60
40
20
Adult
PN28
PN21
PN14
0
100
200
300
400
500
1000
3000
5000
IPI (msec)

Figure 9. The effect of single conditioning pulses on population spike amplitude across ages, expressed as a percentage of baseline amplitude and inter-pulse interval.


PERCENT BASELINE AMPLITUDE
120
100
80
60
40
20
Adult
PN35
PN28
PN14
PN21
0
100
200
300
400
500
1000
3000
5000
IPI (msec)

et al., 1966; Lomo, 1971). At short IPI's, recurrent inhibition masks any facilitation and the test spike is depressed. As the recurrent inhibition decays, facilitation becomes apparent, which then decays itself, by 300-500 msec, back to baseline or to a slight, long-lasting, K^+-mediated depression (Nicoll & Alger, 1981).

The duration and magnitude of depression was greater in immature animals than in adults, and completely masked any facilitation that may have been present. This depression decreased with age, although PN14 and PN21 were reversed in order. The depression was significantly enhanced in younger animals at IPI's up to 500 msec (500 msec IPI, Kruskal-Wallace, $\chi^2=100.9$, df=4, $p < 0.001$).

EXPERIMENT II. THE EFFECT OF HIGH FREQUENCY STIMULATION

STP and LTP develop during narrowly defined time periods in the neocortex (Chapter III). Experiment II was done to describe the postnatal development of STP and LTP in the dentate gyrus.

METHODS

In order to determine the effect of high frequency stimulation on both components of the dentate response, test pulse intensity was set suprathreshold for population spike, 50-80% of maximum. Test pulses were applied at 0.1 Hz throughout the experiment. Following 5 minutes of baseline recording, a single high intensity train was applied to the perforant path, and test pulses continued for 10 minutes. All measures of STP and LTP magnitude were taken following this first train. However, in most animals, two additional trains were applied later to determine reliability of the post-train effects and the effect of

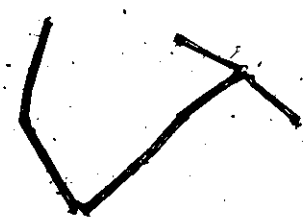
altered train parameters. The standard train was the same as that used for the immature aTCR, 1200 uA at 400 Hz for 100 msec. Subsequent trains varied from 60-400 Hz for 20-800 msec. Both EPSP slope and population spike area were recorded and expressed as a percent of baseline.

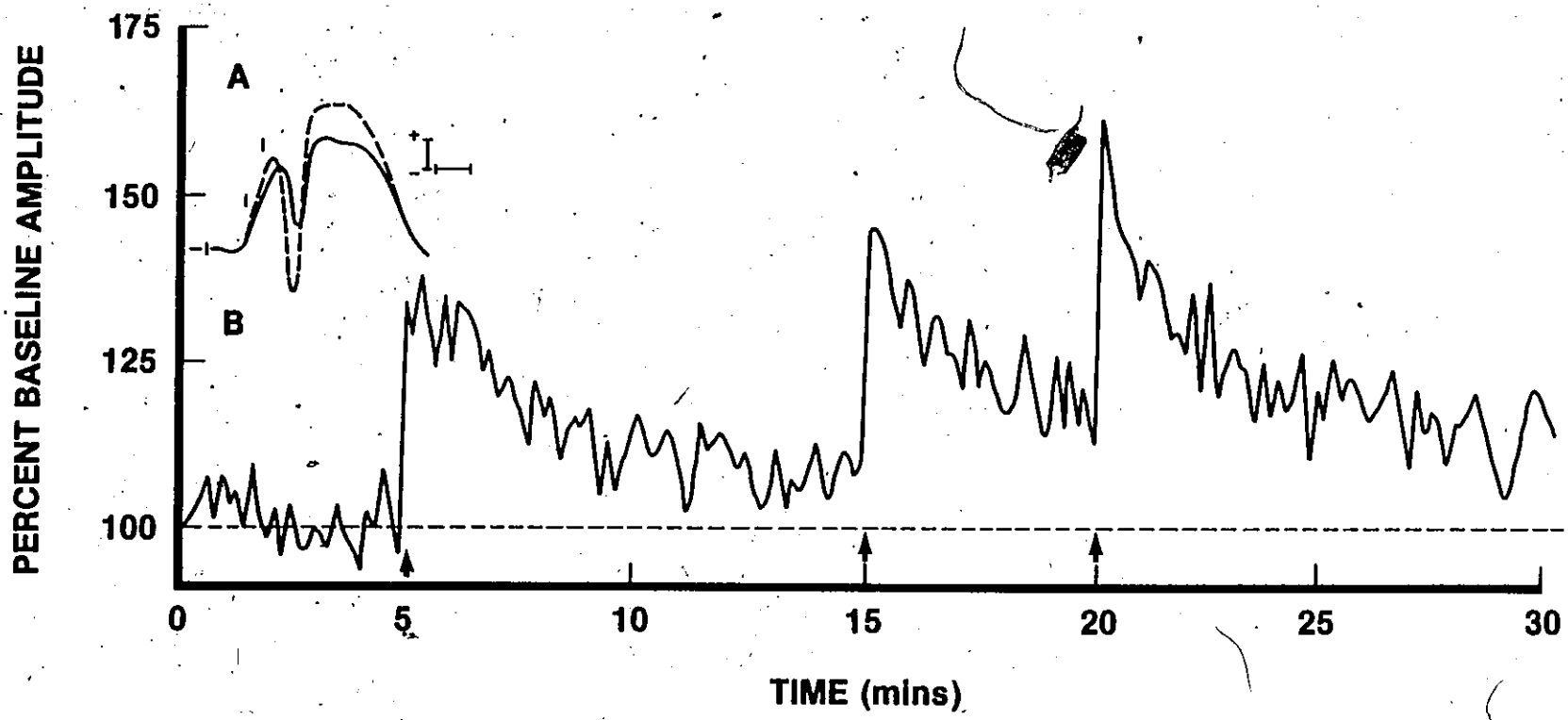
RESULTS

High frequency stimulation of the perforant path in adult animals produced a pronounced and long-lasting potentiation of the EPSP and population spike, as reported elsewhere (Bliss & Lomo, 1973; Douglas & Goddard, 1975). EPSP amplitude preceding and following high frequency stimulation is shown in Figure 10. PAP magnitude was greater for the population spike than for the population EPSP, as has been reported elsewhere (Racine & Milgram, 1983; Racine, et al., 1983). The mean magnitude of STP and LTP of the EPSP were 149±12% and 111±7%, respectively. These values are quite similar to those found for the mature aTCR. Population spike STP and LTP magnitudes were 271±58% and 172±40%, respectively.

As in the neocortex, the incidence of STP and LTP increased with age, as shown in Figures 11 and 12. Figure 11 shows the percent of animals in each age group demonstrating STP and LTP of the EPSP. The increased incidence of STP of the EPSP in animals at PN10, may be due to the relatively high number of animals in this age group with molecular layer recording placements. In many animals of several age groups, molecular layer placements seemed somewhat more sensitive to fluctuations in EPSP amplitude than hilar placements. Furthermore, as was the case in the neocortex, STP in several PN7-PN10 animals consisted

Figure 10. A. Representative dentate response preceding (solid) and following (dashed) high frequency stimulation. Calibration is 2mV and 2 msec. B. Population EPSP amplitude preceding and following high frequency stimulation of the perforant path expressed as a percentage of baseline amplitude. High frequency conditioning trains were applied at the arrows.




PERCENT BASELINE AMPLITUDE
175
150
125
100
A
B
0
5
10
15
20
25
30
TIME (mins)

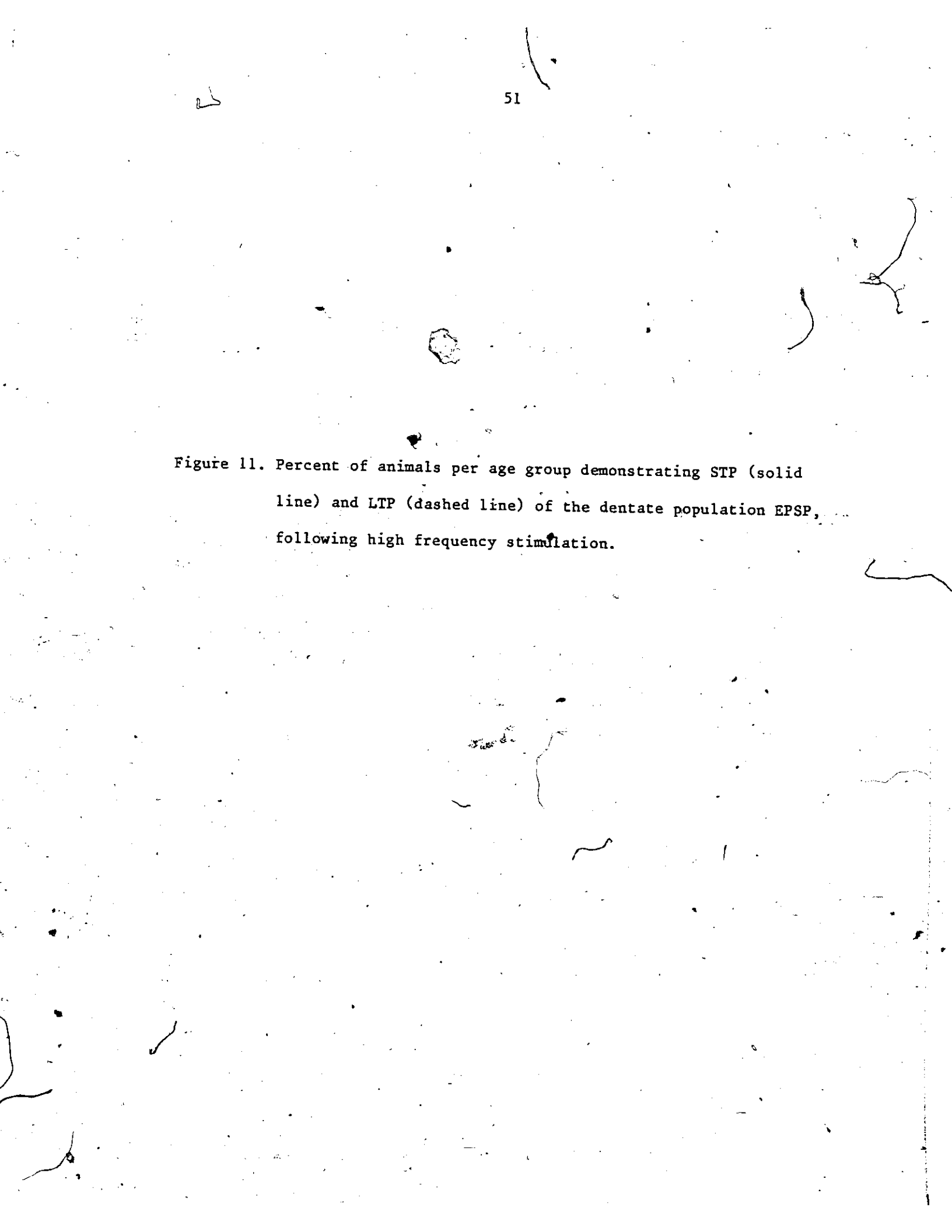


Figure 11. Percent of animals per age group demonstrating STP (solid line) and LTP (dashed line) of the dentate population EPSP, following high frequency stimulation.

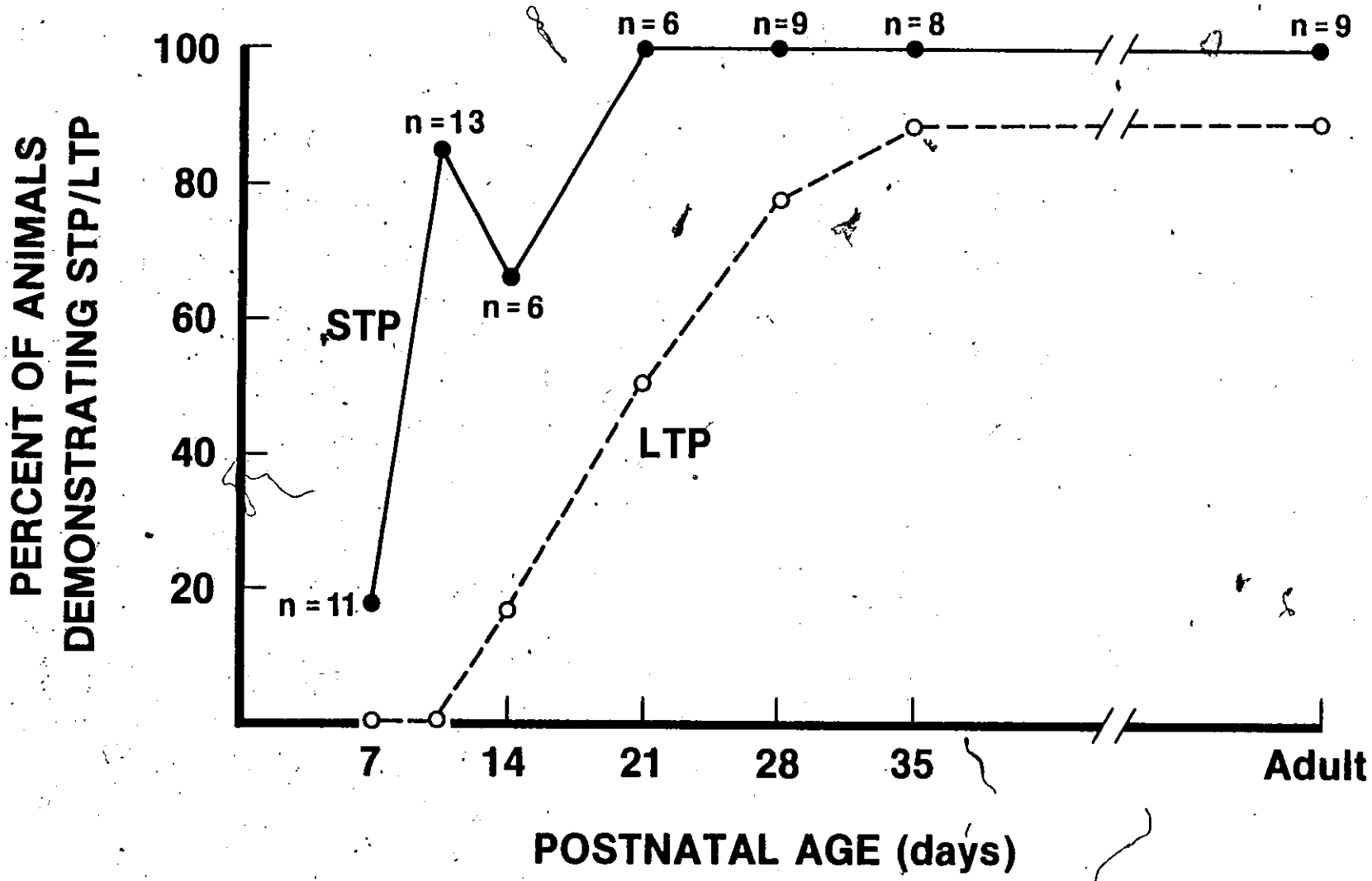
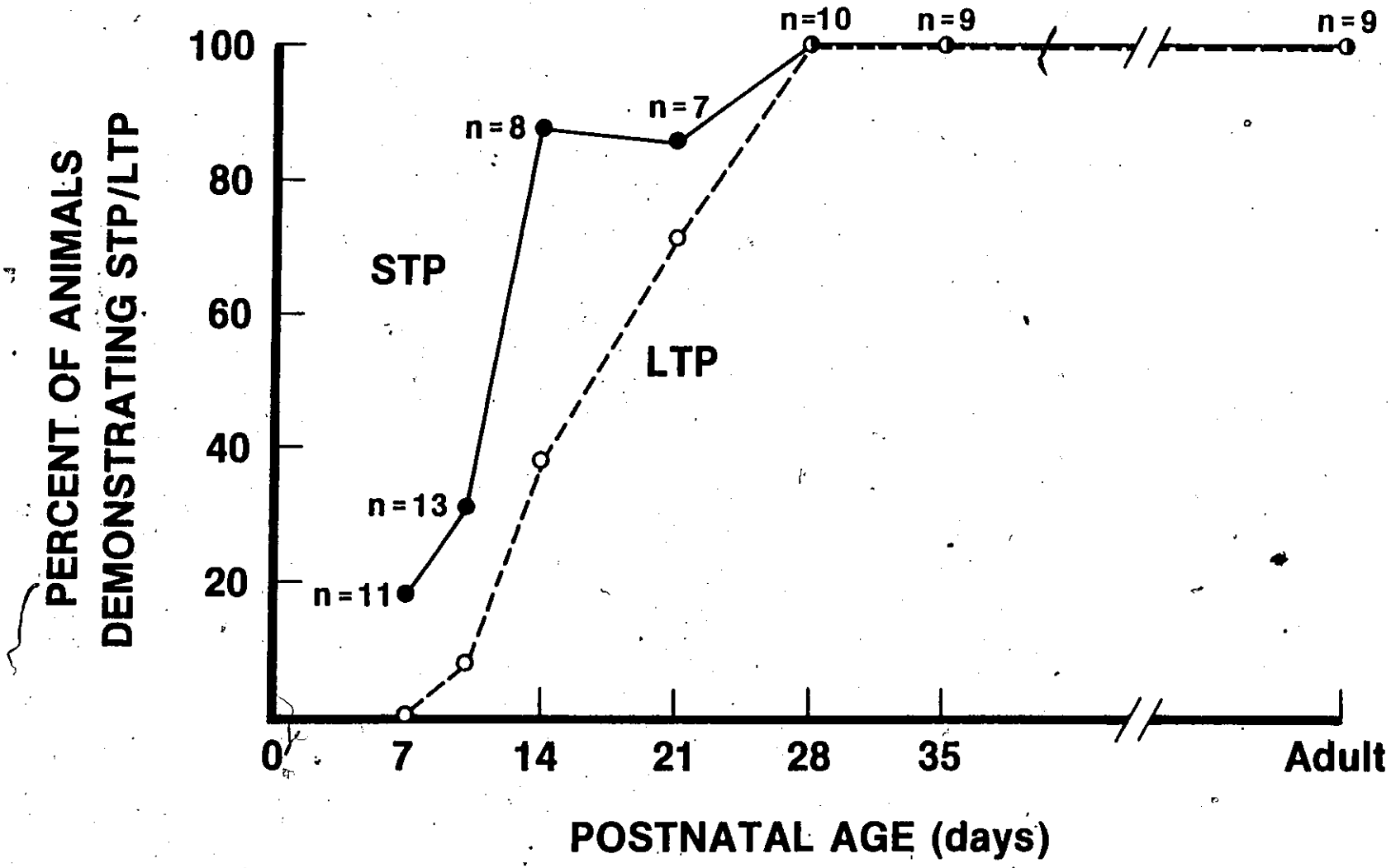

PERCENT OF ANIMALS DEMONSTRATING STP/LTP
100
80
60
40
20
n = 11
n = 13
n = 6
n = 6
n = 9
n = 8
n = 9
STP
LTP
7
14
21
28
35
Adult
POSTNATAL AGE (days)

Figure 12. Percent of animals per age group demonstrating STP (solid line) and LTP (dashed line) of the dentate population spike, following high frequency stimulation.


PERCENT OF ANIMALS
DEMONSTRATING STP/LTP
100
80
60
40
20
n=11
n=13
n=8
n=7
n=10
n=9
n=9
STP
LTP
0
7
14
21
28
35
Adult
POSTNATAL AGE (days)

of only one or two potentiated responses post-train, suggestive of facilitation or augmentation rather than potentiation. Figure 12 shows the percentage of animals per age group demonstrating STP and LTP of the population spike. With both response measures, EPSP and spike, STP appeared several days prior to LTP.

Figure 13 displays population spike amplitude, before and after identical high frequency trains, in several age groups. Representative evoked responses are also displayed. Once present, STP and LTP magnitudes rapidly approached adult levels, between PN14 and PN21.

The decay of STP in the mature dentate could be best described by a single exponential, with time constants ranging from 210-416 seconds. No differences were apparent between immature and mature time constants. In animals of several age groups, responses were monitored for up to 30 minutes post-train to determine the duration of LTP. In all cases, responses potentiated at 10 minutes post-train remained enhanced for the duration of testing. The development of STP and LTP in the dentate gyrus is summarized in Table V.

DISCUSSION

The results of Experiment II suggest that PAP in the dentate gyrus develops during the second postnatal week. The period of dentate PAP development precedes that in the neocortex by several days. As in the neocortex, STP could be detected 3-4 days prior to LTP, and once present, the magnitudes of STP and LTP rapidly approached adult levels.

Dentate PAP development did not correspond well to known structural developments such as synaptogenesis (Crain, et al., 1973) or dendritic spine formation (Duffy & Teyler, 1978). As in the neocortex,

Figure 13. Amplitude of the dentate population spike expressed as a percent of baseline amplitude, preceding and following identical high frequency trains in several age groups. On the right are representative responses for each age group. Calibration is 1mV and 5 msec.

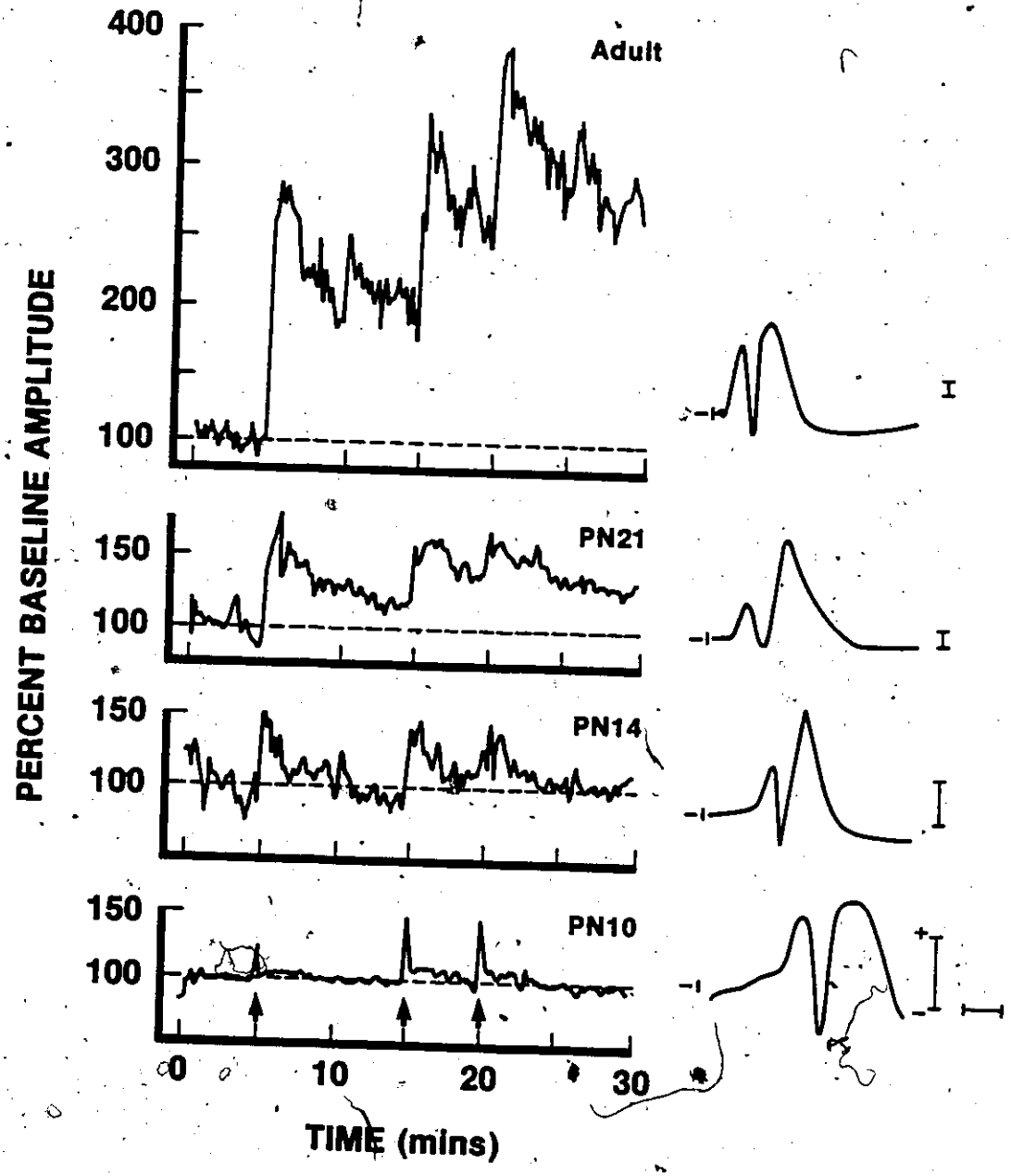

400
300
200
100
Adult
PERCENT BASELINE AMPLITUDE
150
100
PN21
150
100
PN14
150
100
PN10
0
10
20
30
TIME (mins)
I
I
I
I
+
-

TABLE V. Percent demonstrating, and magnitude of, STP and LTP of the population spike and EPSP in the dentate gyrus, in each age group.

TABLE V

Percent demonstrating, and magnitude of, STP and LTP of the EPSP and population spike in the dentate gyrus, in each age group. STP and LTP magnitudes are expressed as percent of baseline, 10 seconds and 10 minutes post-train, respectively (median ± inter-quartile range). Weight (g) is mean ± S.D.

Age (days)	N	Weight (g)	Measure	% Demon. STP	Magnitude STP	% Demon. LTP	Magnitude LTP
7	11	17.3 ±2.3	EPSP	18	100 ±0.0	0	100 ±0.0
			Spike	18	100 ±0.0	0	100 ±0.0
10	5	23.4 3.9	EPSP	100	120 15.0	0	100 0.0
			Spike	80	118 10.0	20	100 0.0
14	12	31.8 5.2	EPSP	44	100 5.0	11	100 0.0
			Spike	83	175 66.5	42	100 10.0
21	10	45.1 9.1	EPSP	100	125 7.5	70	110 5.0
			Spike	90	208 43.5	80	175 45.0
28	16	71.3 17.3	EPSP	100	138 11.0	80	111 7.5
			Spike	100	158 39.5	94	125 19.5
35	14	119.9 15.1	EPSP	100	150 12.0	92	115 5.0
			Spike	100	319 39.0	100	185 47.0
Adult	12	357.5 74.5	EPSP	100	148 3.5	92	110 5.0
			Spike	100	270 35.0	100	170 27.0

therefore, neurochemical maturation may be the critical factor underlying PAP development in the dentate gyrus.

Facilitation in the immature dentate was completely masked by a long-lasting depression following the conditioning pulse. This depression decreased in magnitude and duration with increased age. This long-lasting depression may be due to the increased barbiturate sensitivity of the younger animals. The next experiments examine the postnatal development of PAP in non-barbiturate anesthetized animals.

CHAPTER V. DEVELOPMENT OF PAP IN THE DENTATE GYRUS OF URETHANE ANESTHETIZED RATS.

The sensitivity of the CNS to barbiturates has been shown to vary inversely with age (Bianchine & Ferguson, 1967; Jondorf, Maikel & Brodie, 1959; Kalser, Forbes & Kunig, 1969; King & Becker, 1963; Mirkin, 1970; Staudacherova, Mares & Trojan, 1979). In order to ensure that the development of PAP, as described in the previous experiments, is not due to the differential sensitivity of young rats to the pentobarbital anesthetic used, the present experiment was an examination of PAP development in the dentate gyrus of urethane-anesthetized rats.

Urethane was chosen as the alternate anesthetic for the present study because of distinct differences between the actions of barbiturates and urethane. Barbiturates have several pre- and post-synaptic actions. Barbiturates decrease excitatory transmitter output (Richards, 1972; Weakly, 1969), and increase pre- (Eccles, Schmidt & Willis, 1963) and post-synaptic inhibition (Nicoll, Eccles, Oshima & Rubia, 1975; Scholfield, 1980). The potentiation of post-synaptic inhibition appears to be mediated via the GABA-ergic system (Higashi & Nishi, 1982). Urethane, although not studied as extensively as barbiturates, appears to block the action of excitatory amino acid neurotransmitters, post-synaptically (Evans & Smith, 1982). Urethane has only minor effects on inhibition (Scholfield, 1980) and on the GABA-ergic system (Evans & Smith, 1982).

METHODS

The methods used here were identical to those described in the previous chapter for single conditioning pulse and high frequency stimulation. All animals, regardless of age or weight were given the same dosage of urethane I.P., 2 g/kg.

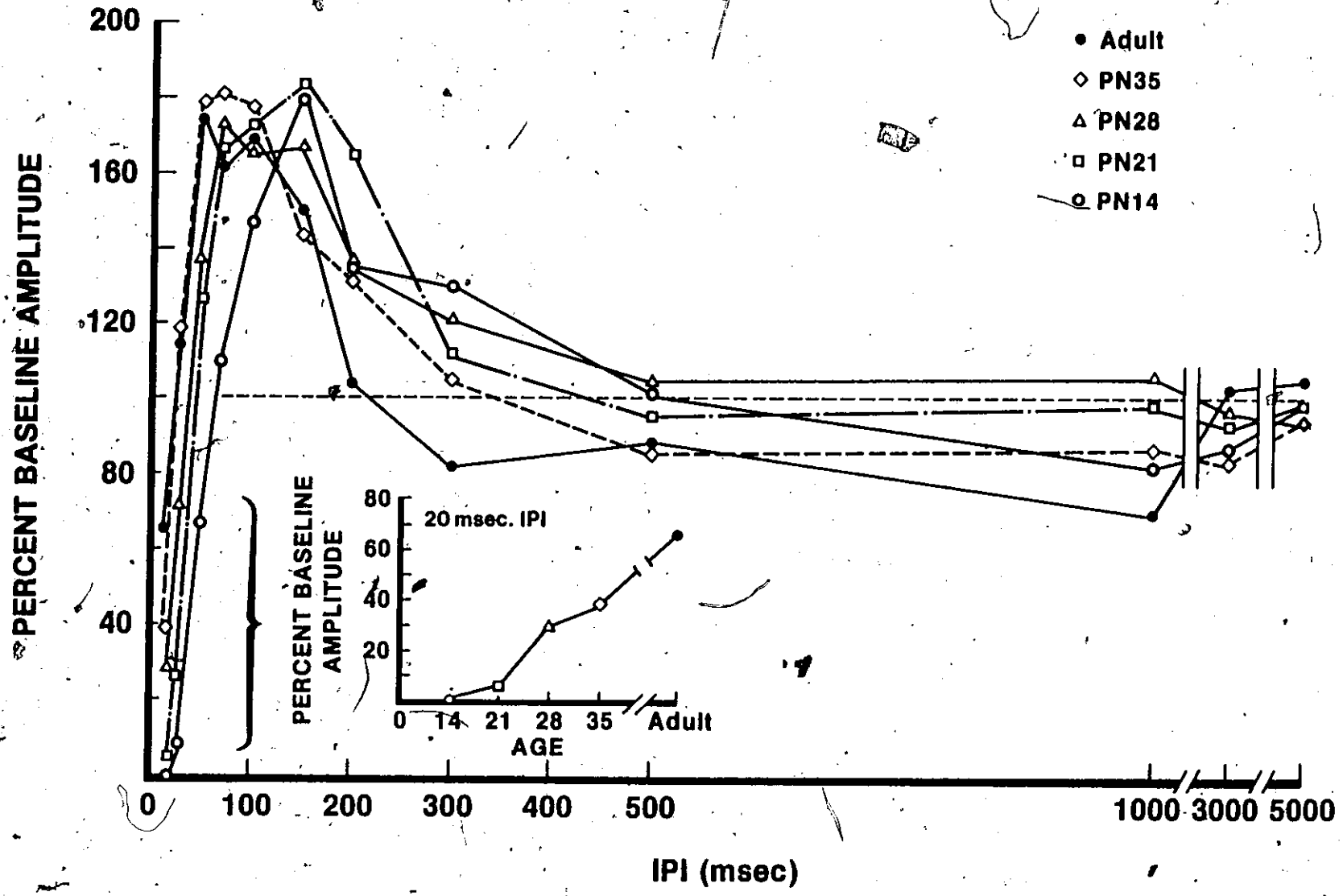
RESULTS

Responses evoked in the dentate hilus to perforant path stimulation appeared identical to those seen in barbiturate-anesthetized animals. Response thresholds and maximal response amplitudes did not significantly differ between urethane-anesthetized rats and their appropriately aged, pentobarbarbital anesthetized controls from Chapter IV. These animals did, however, appear more susceptible to AD's during electrode implantation. Data from animals with unusually prolonged AD's were eliminated from the present results.

The effect of single conditioning pulses on subsequent test spikes, across age, is shown in Figure 14. In contrast to the prolonged depression seen in immature, barbiturate-anesthetized rats, all age groups demonstrated a pronounced facilitation of the population spike. The peak magnitude of facilitation did not differ with age, varying from 170-185%. This facilitation decayed by 300-500 msec. In all ages, facilitation was preceded by an initial depression, presumably mediated by recurrent inhibition (Andersen, et al., 1966). The magnitude of this depression was greater in younger animals at short IPI's (e.g., 20 msec IPI, Kruskal-Wallace, $\chi^2=18.01$, $df=4$, $p < 0.005$).

The incidence and magnitude of STP and LTP in urethane-

Figure 14. The effect of single conditioning pulses on population spike amplitude across ages, expressed as a percentage of baseline amplitude and inter-pulse interval, in urethane anesthetized animals. The inset shows the test spike amplitude at 20 msec IPI, as a function of age. Note the increased depression in younger animals at this IPI.


200
160
120
80
40
PERCENT BASELINE AMPLITUDE
Adult
PN35
PN28
PN21
PN14
0
100
200
300
400
500
1000
3000
5000
IPI (msec)
PERCENT BASELINE AMPLITUDE
80
60
40
20
20 msec. IPI
0
14
21
28
35
Adult
AGE

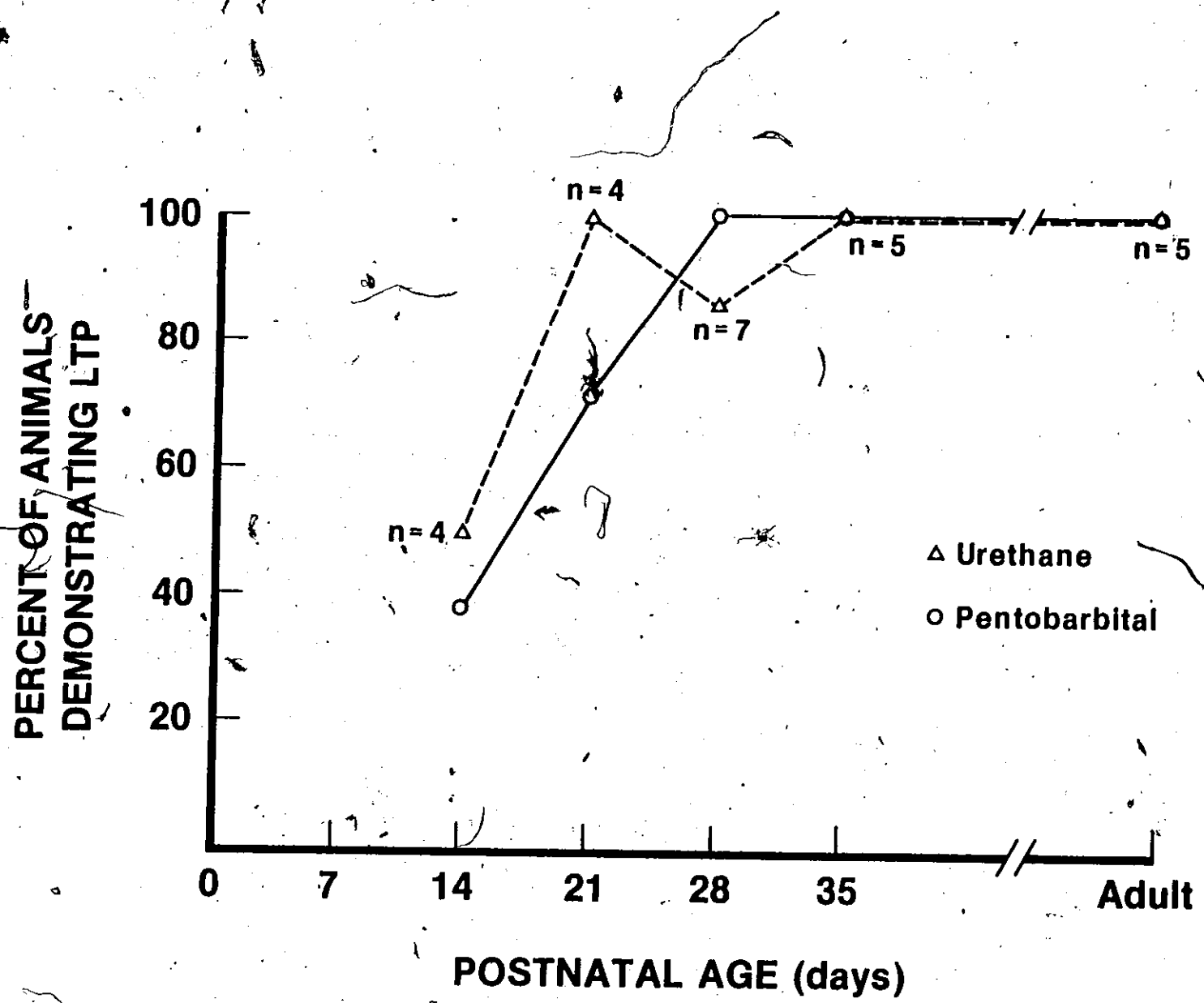
anesthetized animals did not differ from that reported in Chapter IV. Figure 15 displays the percent of animals in each age group demonstrating LTP of the population spike under urethane and pentobarbital anesthesia. Within age group comparisons of the magnitude of STP and LTP, showed no significant differences between barbiturate and urethane treated groups (Mann-Whitney U, $p > 0.10$).

DISCUSSION

The present results demonstrate that the postnatal maturation of STP and LTP in the dentate gyrus, described in Experiment III, is not an artifact of the greater sensitivity of young animals to barbiturate anesthesia. On the other hand, paired-pulse effects were completely different in the presence of barbiturate versus non-barbiturate anesthetic. The long-lasting paired-pulse depression seen in immature barbiturate-anesthetized animals (Chapters III and IV) was replaced by a short-lasting depression followed by facilitation. The peak magnitude of facilitation was the same across age groups.

The magnitude of the initial depression, however, was greater in younger animals. This suggests either, 1) a small, age-dependent effect of urethane on inhibition, 2) increased susceptibility in young animals to transmitter depletion, or 3) greater recurrent inhibition in younger animals. The first possibility cannot be entirely dismissed. There did not, however, appear to be any increased behavioral sensitivity to urethane in young animals; e.g., the same dose level produced surgical anesthesia in all age groups. Furthermore, as stated above, urethane is reported to have only minor effects on inhibition (Evans & Smith, 1982; Scholfield, 1980). The second possibility, increased transmitter

Figure 15. Percent of urethane (dashed line) and pentobarbital (solid line) anesthetized animals, per age group, demonstrating LTP of the population spike following high frequency stimulation.


PERCENT OF ANIMALS DEMONSTRATING LTP
100
80
60
40
20
0
7
14
21
28
35
Adult
POSTNATAL AGE (days)
n = 4
n = 4
n = 7
n = 5
n = 5
Urethane
Pentobarbital

depletion, is unlikely because the magnitude of facilitation was essentially identical across all ages. If less transmitter were available for release, less facilitation might be expected (Zucker, 1974). This leaves the third possibility, greater inhibition in young animals. This increased inhibition may be either GABA-mediated or K^+-mediated (Sato, Austin & Yai, 1967). If true, these results are in apparent contradiction to in vitro results suggesting late development of inhibition in the hippocampal formation (Harris & Teyler, 1983b; Schwartzkroin, 1982; Schwartzkroin, et al., 1982).

CHAPTER VI. DEVELOPMENT OF PAP IN THE DENTATE GYRUS OF UNANESTHETIZED RATS

The results of the previous experiments, in anesthetized rats, demonstrate that STP and LTP develop during a one to two week postnatal period. The present experiment was done to extend this finding to unanesthetized rats. Recording from awake animals with chronic, indwelling electrodes eliminates problems associated with acute preparations such as fluctuating anesthesia level, depressed respiration and body temperature, and, as seen in Chapters IV and V, differential sensitivity to anesthetics. In addition, the use of chronically prepared animals allows multiple testing of individual rats over several days. It was therefore hoped that the postnatal maturation of PAP in the dentate gyrus could be traced in single animals, providing a more detailed description of PAP development, than could be obtained from group comparisons.

METHODS

Only animals aged PN10 to adult were used here. Animals were anesthetized with either sodium pentobarbital (65 mg/kg, adults) or sodium methohexital (18 mg/kg, PN10-PN35). Electrodes were implanted in the dentate hilus and perforant path as described above and cemented in place with dental acrylic (Nuweld). Ground leads were attached in one of two ways. In more mature animals (PN28-adult) the ground lead was attached to a screw mounted above the posterior neocortex/cerebellum. In younger animals (PN10-PN21) a bare wire was threaded through two

holes drilled above the posterior neocortex such that the wire passed between the dura and the skull.

Animals anesthetized with pentobarbital were allowed at least one week of recovery before data collection. Neonatal chronics anesthetized with methohexital were allowed at least 4-24 hours of recovery. Methohexital is a fast-acting barbiturate, providing surgical levels of anesthesia for 10-25 minutes in neonates with the dosage used here. These animals appear behaviorally normal within 1-3 hours after injection. See Appendix I for a justification of this procedure. Animals not tested on the same day as surgery were returned to their litters.

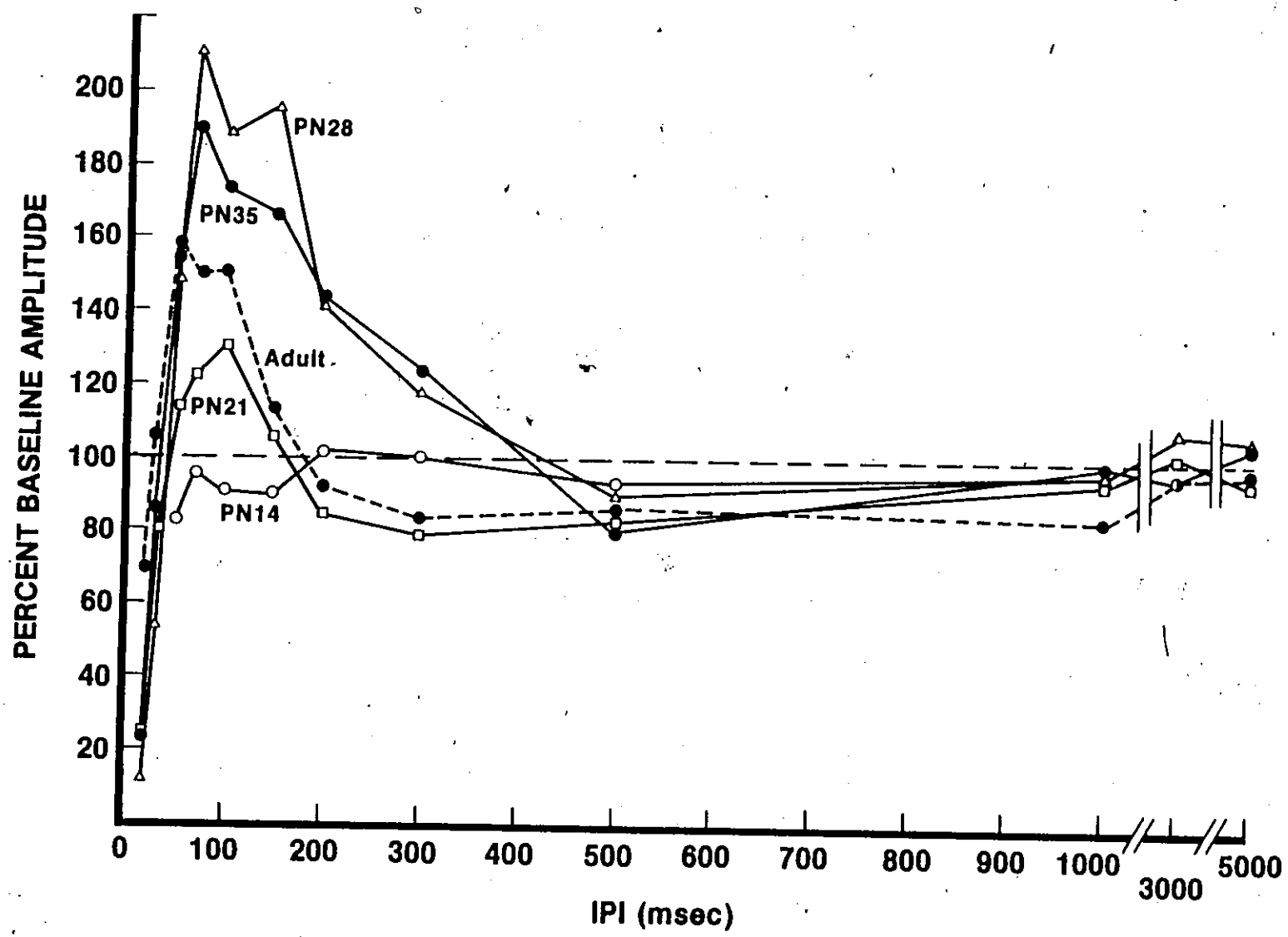
PAP tests were done as described previously. An attempt was made to follow the development of PAP in single animals between PN10 and PN14. Unfortunately, due to technical difficulties, responses could not be maintained in these young animals for more than a few hours, thus daily testing was unsuccessful.

RESULTS

Responses in awake animals between PN10 and PN21 were very difficult to maintain and were generally quite variable. In addition, response amplitude in young animals appeared more influenced by behavioral state than in adult animals (Leung, 1980; Winson & Abzug, 1978). Due to the difficulty and variability of the preparation, the results presented here will be somewhat more qualitative than in the previous experiments.

The effect of single conditioning pulses on subsequent test population spike amplitude, across ages, is shown in Figure 16.

Figure 16. The effect of single conditioning pulses on population spike amplitude across ages, expressed as a percentage of baseline amplitude and inter-pulse interval, in unanesthetized animals.


PERCENT BASELINE AMPLITUDE
200
180
160
140
120
100
80
60
40
20
PN28
PN35
Adult
PN21
PN14
0
100
200
300
400
500
600
700
800
900
1000
3000
5000
IPI (msec)

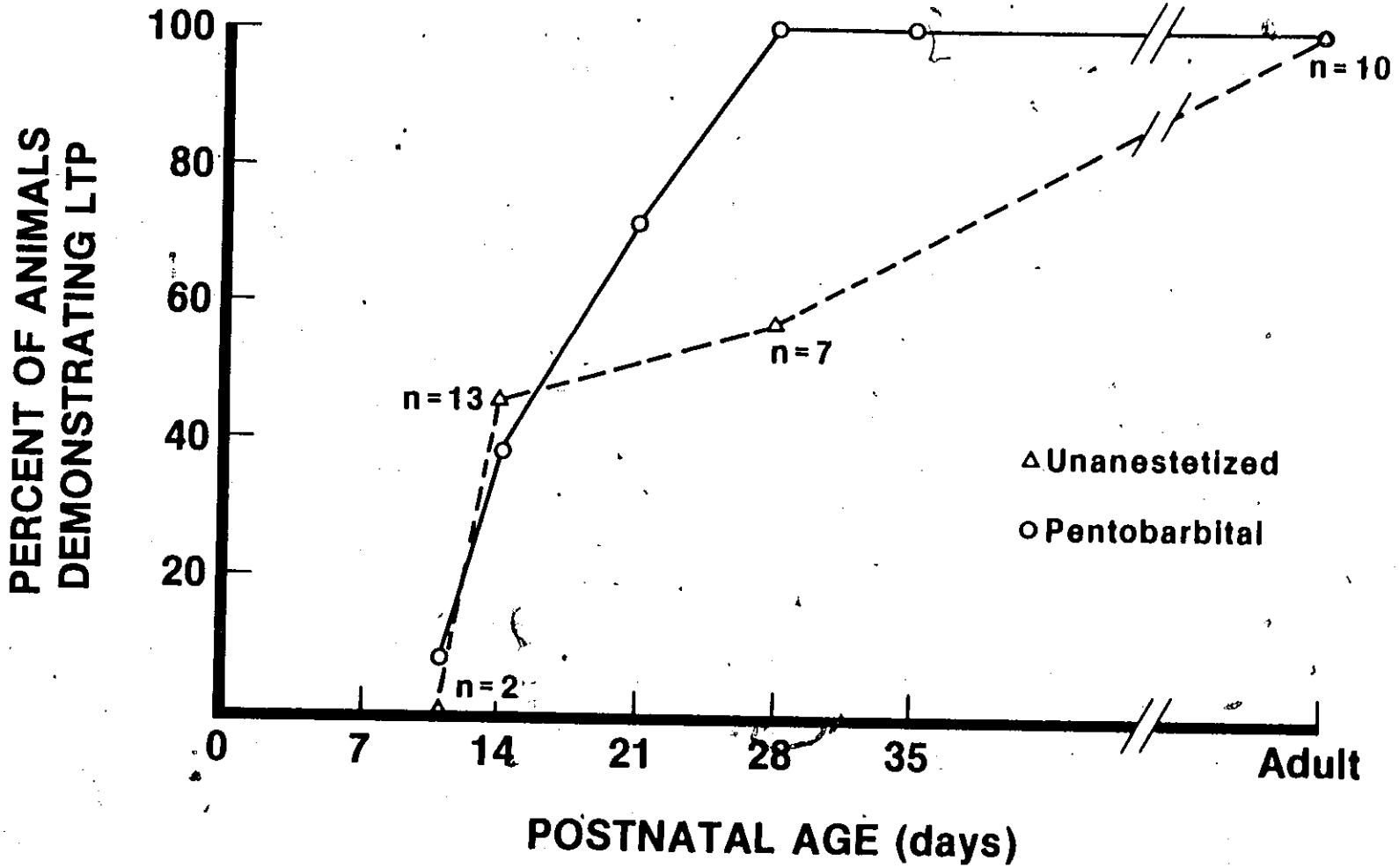
Although more variable, paired-pulse effects in awake animals, PN21-adult, were similar to those found in urethane-anesthetized animals. Following an initial depression at short IPI's, test responses were facilitated for 200-300 msec. While PN14 animals, as a group, did not demonstrate facilitation, 3 of 7 individuals did demonstrate clear facilitation of the population spike, peaking at up to 180% of baseline.

The postnatal development of STP and LTP in unanesthetized animals was similar to that reported in anesthetized animals. STP and LTP were not reliably observed until around PN14. Figure 17 shows the development of LTP in the unanesthetized dentate. This Figure includes LTP measures of both the population EPSP and spike, since population spikes were not always observed in PN10-PN14 animals. Although the percentage of chronic PN28 animals demonstrating LTP (4/7) was low compared to pentobarbital treated PN28 animals, the general trend of a sudden onset of LTP around PN14 is apparent. In several animals of different ages, response amplitude was monitored for up to 30 minutes post-train. Responses that were still potentiated at 10 minutes post-train (the operational definition of LTP used here) remained enhanced for the duration of testing.

DISCUSSION

The results of the present experiment demonstrate that the postnatal development of PAP in the dentate gyrus can be observed in unanesthetized, awake animals and its development is, therefore, not an artifact of the anesthetized immature CNS. LTP could first be detected on PN14.

Figure 17. Percent of unanesthetized (dashed line) and pentobarbital anesthetized (solid line) animals, per age group, demonstrating LTP of the population spike following high frequency stimulation.


PERCENT OF ANIMALS DEMONSTRATING LTP
100
80
60
40
20
0
7
14
21
28
35
Adult
POSTNATAL AGE (days)
n = 13
n = 2
n = 7
n = 10
Unanestetized
Pentobarbital

Paired-pulse tests demonstrated that facilitation could be produced in all age groups. Although these data were quite variable, they correspond well with those found with urethane anesthetized animals, i.e., younger animals demonstrated greater depression.

CHAPTER VII. GENERAL DISCUSSION

The present series of experiments have demonstrated that post-activation potentiation of synaptic efficacy develops during a narrowly defined time period in the rat dentate gyrus and neocortex. Similar findings have been reported in the in vitro rat hippocampal CA1 (Baudry, et al., 1981; Harris & Teyler, 1982) and dentate (Duffy & Teyler, 1978b). Furthermore, the age at which PAP can first be detected is dependent on the structure involved. STP and LTP develop near the end of the second postnatal week in the dentate gyrus and during the third postnatal week in the neocortex. Although the dentate gyrus also matures structurally prior to the neocortex, no strong correspondence was found between known development of specific neural structures, such as synaptogenesis or spine formation, and STP/LTP development in either system.

Electrophysiological research on immature animals presents several unique problems. For example, the increased sensitivity of immature rats to barbiturate anesthetic had a major influence on the results of paired-pulse tests (see Chapters IV and V). This confound was eliminated by replicating single pulse and high frequency conditioning stimulation tests in urethane-anesthetized and awake animals. Testing of awake animals further eliminated, or reduced, such problems as decreased body temperature and anoxia during anesthetization. It was possible, of course, that immature synapses were capable of supporting STP/LTP, but that STP and LTP thresholds were simply higher in immature systems. For example, immature axons are

known to have very long refractory periods (Crepel, 1974), and may be incapable of following brief, high frequency conditioning trains. Therefore, these brief trains may be ineffective in sufficiently activating the synapse to produce STP/LTP. The extreme variations in conditioning train parameters used in Chapters III and IV (from 10-400 Hz for 50 msec-10 seconds), however, make this explanation unlikely, and indicates that immature synapses are indeed incapable of supporting STP and LTP. (However, it should be noted that Duffy and Teyler [1978] reported STP [but not LTP] of the EPSP in the in vitro rat dentate at PN7 following four 15 Hz, 10 second trains, applied to the perforant path.)

Another explanation for the late development of STP is an increased susceptibility to transmitter depletion in young animals. There are fewer pre-synaptic vesicles in immature than in mature neurons (Johnson & Armstrong-James, 1970; Schwartzkroin & Kunkel, 1982), and thus presumably, less transmitter available for release. The mechanism for potentiation may thus be functional at immature synapses, but may be counteracted by a train-induced depletion. Ohmori and colleaques (Ohmori, 1982; Ohmori, Rayport & Kandel, 1981) addressed this possibility in their study of the development of PTP (STP) in Aplysia. They decreased pre-synaptic transmitter output by hyperpolarizing the pre-synaptic neuron, and found that immature synapses were still incapable of demonstrating PTP.

The lack of correspondence between PAP development and neural structural developments suggests neurochemical maturation may be the critical factor. STP, as stated in the Introduction, is dependent on

accumulation of Ca^{++} in the pre-synaptic nerve terminal during the conditioning train (Magleby & Zengel, 1975; Magleby & Zengel, 1976a). Ca^{++} appears capable of entering the pre-synaptic terminal in all age groups tested in the present studies since post-synaptic potentials were recorded in all age groups. However, the increase in transmitter release following conditioning may not depend directly on the increased Ca^{++} concentration, but rather indirectly on some, as yet undefined, Ca^{++}-activated factor such as protein phosphorylation (Castellucci, Kandel, Schwartz, Wilson, Nairn & Greengard, 1980; Zengel & Magleby, 1982), or a Ca^{++} binding protein (e.g., calmodulin; Turner, Baimbridge & Miller, 1982). The postnatal development of STP, then, may depend on the development of this factor after initial synaptic contact has been made (Ohmori, 1982; Ohmori, et al., 1981).

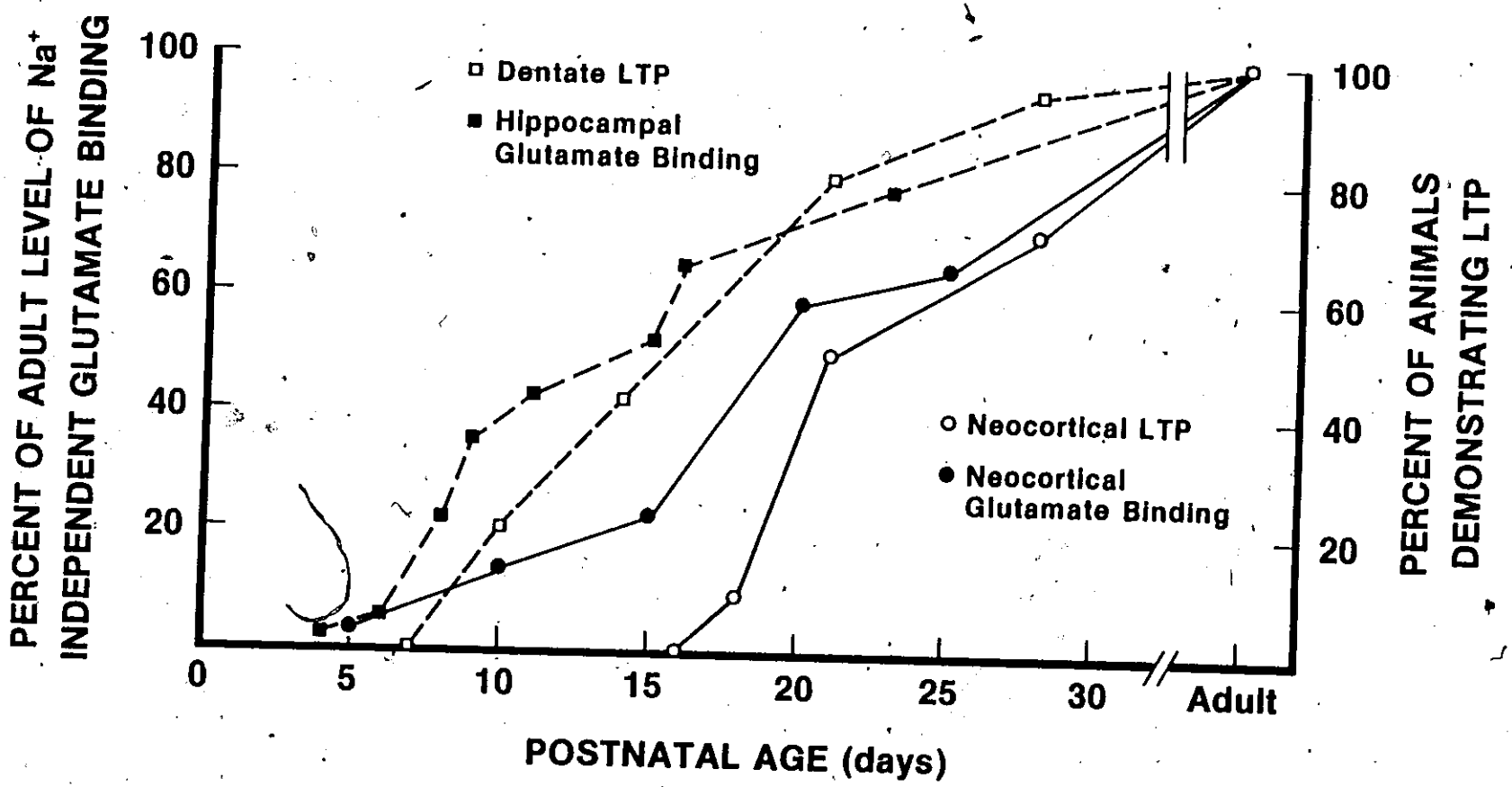
The lack of correspondence between LTP and structural development, especially dendritic spine formation, is surprising in light of several studies suggesting changes in spine morphology as a mechanism of LTP (Desmond & Levy, 1983; Lee, et al., 1980; Van Harreveld & Fifkova, 1975). However, correspondence between LTP and structural development was determined here by visual inspection of graphs depicting the development of specific neural structures, e.g. the number of spines/dendrite at several postnatal ages. It was hoped that comparison of these graphs with an LTP development graph (e.g. Figure 11) would result in a "close match", e.g., the age at first appearance and attainment of adult levels of dendritic spines would correspond closely to the age at first appearance and attainment of adult levels of LTP. The functional relationships, however, may be non-linear, in which case

a close match of developmental curves would not be seen. Nevertheless, a better fit between phenomena and substrate could provide insight into underlying mechanisms.

Another hypothesized mechanism of LTP is a Ca^{++}-activated increase in post-synaptic glutamate binding sites (Baudry & Lynch, 1980a). Evidence in favor of this hypothesis is: 1) Glutamate receptor number in purified synaptic membrane is increased by low levels (10-50 uM) of Ca^{++} (Baudry, Kramer & Lynch, 1983; Baudry & Lynch, 1979; Lynch, et al., 1982). 2) Intense, LTP-producing, synaptic activation produces an increase in glutamate receptor number (Baudry, et al., 1980; Lynch, et al., 1982). 3) The induction of LTP requires the presence of extracellular Ca^{++} (Dunwiddie & Lynch, 1979; Dunwiddie, et al., 1978). 4) LTP can be selectively blocked in single neurons by intracellular injections of EGTA, a Ca^{++} chelator, into those neurons (Lynch, et al., submitted). Finally, of most importance to the present discussion, 5) the postnatal development of LTP in the hippocampal CA1 region closely parallels the ontogeny of calcium's stimulant effect on glutamate receptors in that region (Baudry, et al., 1981a).

If Ca^{++}-stimulation of glutamate receptors is the mechanism of LTP, then the development of Ca^{++}-stimulation should correspond to LTP development in both the hippocampal formation and the neocortex. Although development of Ca^{++} effects on glutamate receptors has not been examined in the neocortex, development of the receptor sites themselves might be considered an appropriate approximation. Figure 18 displays the postnatal development of Na-independent glutamate binding (Baudry,

Figure 18. Postnatal development of LTP and Na-independent glutamate binding sites in the neocortex and dentate gyrus. Glutamate binding expressed as a percent of adult level. Neocortical glutamate binding adapted from Sanderson & Murphy (1982). Hippocampal glutamate binding (whole hippocampus) adapted from Baudry, et al. (1981a). Neocortical and dentate LTP adapted from Figures 6 and 12, respectively.


PERCENT OF ADULT LEVEL OF Na+ INDEPENDENT GLUTAMATE BINDING
100
80
60
40
20
0
5
10
15
20
25
30
Adult
POSTNATAL AGE (days)
Dentate LTP
Hippocampal Glutamate Binding
Neocortical LTP
Neocortical Glutamate Binding
PERCENT OF ANIMALS DEMONSTRATING LTP

et al., 1981a; Sanderson & Murphy, 1982) and LTP (Chapters III and IV) in the rat neocortex and dentate gyrus. Neocortical glutamate binding development lags hippocampal binding by several days as does LTP development. This close correspondence of glutamate binding and LTP in both the hippocampal formation and the neocortex suggests a possible role for glutamate receptors in LTP, and might be taken as support for the Baudry-Lynch (1980) LTP mechanism hypothesis. An examination of the postnatal development of Ca^{++} effects on glutamate binding in the neocortex is suggested.

As reported here and elsewhere (Baudry, et al., 1981a; Duffy & Teyler, 1978; Harris & Teyler, 1982), PAP develops relatively late in the postnatal hippocampal formation and neocortex. Neonatal rats, however, appear to be capable of learning simple tasks prior to PAP development in these systems (Caldwell & Werboff, 1962; Campbell & Coulter, 1976; Johanson & Hall, 1982; Misanin, Nagy, Keiser & Bowen, 1971; Ray & Nagy, 1979; Thoman, Wetzel & Levine, 1968). The majority of these neonatal learning studies involve simple, reflexive tasks, e.g. escape behaviors, and may therefore be dependent on earlier developing, sub-cortical systems.

It is interesting to note, however, that at least one study reported that long-term memory (24 hours) developed several days after short-term memory, around PN9 (Misanin, et al., 1971). This is similar to the relationship reported here for the development of STP and LTP, and near the time of first detection of LTP in the dentate (Chapter IV) and CA1 (Baudry, et al., 1981a; Harris & Teyler, 1982). Furthermore, rats as young as PN1 can learn taste aversions following pairing of

ingestion of a novel flavor and subsequent toxicosis, as long as there is no delay between ingestion and illness (Gemberling & Domjan, 1982). Mature rats are capable of forming taste aversions with delays of several hours between ingestion and illness. Long-delay learning requires maintainence of the stimulus trace (novel flavor) until the consequence of that stimulus occurs (illness). The ability to form long-delay taste aversions develops between PN12 and PN15 in rats (Gregg, Kitrell, Domjan & Amsel, 1978), which, again, is near the time of LTP development in the hippocampus and neocortex. A closer examination of synaptic functional development and correlation with behavioral development may prove fruitful in determining the neural substrates of memory processes.

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APPENDIX I: THE TIME COURSE OF ELECTROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF SODIUM METHOHEXITAL (BRIETAL)

Sodium Methohexital (Breital) is a highly lipid-soluable, fast-acting barbiturate (Sharpless, 1970). As shown in this thesis and elsewhere (Nicoll, et al., 1975; Scholfield, 1978) barbiturates increase the magnitude and duration of GABA-mediated inhibitory post-synaptic potentials. The present study used this fact, along with behavioral observation, to determine the time course of sodium methohexital effects on the CNS. A description of the duration of methohexital CNS action was important in determining the appropriate recovery time for neonates implanted with chronic indwelling electrodes while under methohexital anesthesia. To allow for any maintainance doses required during normal surgery, a single dosage, approximately twice normal, was given here.

METHODS

Animals, aged PN14-PN28, were implanted with chronic, indwelling, stimulating and recording electrodes in the perforant path and dentate gyrus, respectively, as described elswhere in this thesis, and allowed to recover.

Paired-pulses, were applied once every 20 sec for one hour. The conditioning pulse was suprathreshold for population spike and inter-pulse interval was 100 msec. Five to ten minutes after the start of the paired-pulse series, a single intra-peritoneal injection of sodium methohexital (18-50mg/kg) was given and paired-pulse testing continued. Responses were stored and analysed as described above. The amplitude of the population spike to the test pulse, expressed as a percentage of the

conditioning spike, was used as a measure of paired-pulse facilitation/depression.

Behavioral activity was classified in one of four categories: 1) mobile, 2) immobile awake, 3) loss of posture if pushed by experimenter or staggers if mobile, 4) loss of righting reflex. Behavioral analysis was done blind with regard to electrophysiological data.

RESULTS

Methohexital produced a pronounced increase in the duration of inhibition, as evidenced by a masking of paired-pulse facilitation of the population spike by paired-pulse depression, in all age groups. The onset of depression was rapid in all animals but it's duration varied with age (Figure 1).

In all cases, the behavioral effects of anesthesia appeared sooner than, and outlasted any apparent electrophysiological effects. The onset of behavioral anesthesia occurred within 1 minute post-injection. Within 2 minutes post-injection, the test population spike became depressed. This paired-pulse depression lasted from 10 minutes to approximately 2 hours, depending on age. Behavioral anesthesia generally lasted 10-20 minutes longer than any noticable electrophysiological depression. With the electro-physiological and behavioral measures used here, all animals were completely recovered by 90-180 minutes post-injection.

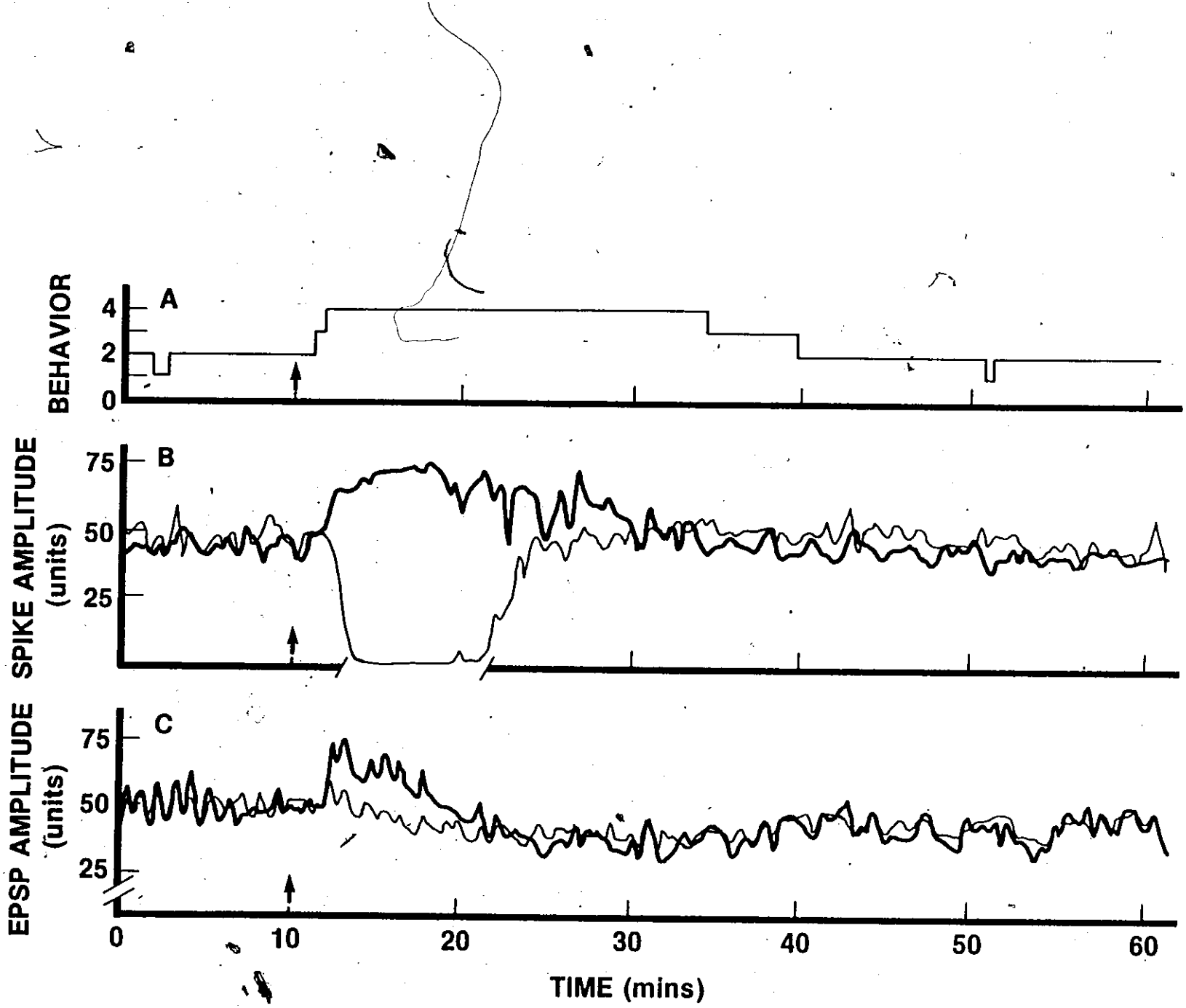
Following injection, although the test response showed only depression, the conditioning response occassionaly demonstrated a biphasic shift in amplitude. Immediately post-injection, population

Figure 1. The electrophysiological and behavioral effects of sodium methohexital on a single PN28 rat.

A. The behavioral state of the rat before and after injection of 49 mg/kg methohexital I.P. Numbers refer to behavioral condition as outlined in the text.

B. Amplitude of the population spike response to the conditioning (thick line) and test (thin line) pulses before and after methohexital injection.

C. Amplitude of the EPSP response to the conditioning (thick line) and test (thin line) pulses before and after methohexital injection.


A
BEHAVIOR
4
2
0
B
SPIKE AMPLITUDE (units)
75
50
25
C
EPSP AMPLITUDE (units)
75
50
25
0
10
20
30
40
50
60
TIME (mins)

spike and epsp amplitude increased. This increase in amplitude was generally brief (2-5 minutes) and was followed by a gradual depression, which lasted approximately as long as the depression of the test pulse. This biphasic change in conditioning pulse amplitude appeared to be dose-dependent, being more pronounced with higher doses. A detailed analysis was not done.

DISCUSSION

The duration of behavioral and electrophysiological effects of sodium methohexital varied inversely with age. Behavioral anesthesia had a faster onset and longer duration than the electrophysiological effects measured here. In all cases, however, animals were completely recovered (according to the criteria above) within 3 hours. These results suggest therefore, that a 4-24 hour recovery time following methohexital anesthesia in neonates should be sufficient to avoid confounding anesthetic effects.

APPENDIX II. CALCIUM-INDUCED LONG-TERM POTENTIATION IN THE ADULT AND IMMATURE HIPPOCAMPUS, IN VITRO.

Long-term potentiation following brief exposure to high extracellular calcium levels has been reported in the hippocampal CA1 region of mature rats (Turner, et al., 1982). Calcium-induced LTP is presumed to have a similar (or the same) mechanism as LTP induced by high frequency stimulation (Turner, et al., 1982). The present experiment was an attempt to determine if calcium-induced LTP could be demonstrated in the immature hippocampal CA1 region, prior to the age at which LTP can be produced by high frequency stimulation. Production of LTP with high calcium should avoid such problems as long refractory periods, and thus inability to follow high frequency stimulation, in immature neurons.

To allow a direct comparison of mature and immature preparations, hippocampal slices from adult and immature rats were placed in the same chamber, and all manipulations were identical and simultaneous for both slices. Immature rats were aged PN9-PN10, the age at which stimulation-induced LTP first begins to appear in the CA1 (Baudry, et al., 1981; Harris & Teyler, 1982).

METHODS

Hippocampal slices from adult and PN9-PN10 rats were prepared and maintained in a perfusion chamber as described by Schwartzkroin (1981). Slices were perfused with a medium containing 124 mM NaCl, 3 mM KCl, 2 mM $MgSO_4$, 26 mM $NaHCO_3$, 2 mM CaCl, and 10 mM Dextrose. The

medium was oxygenated with 95% O2 and 5% CO2. The 4mM CaCl was intoduced via a three-way valve, as described by Turner, et al. (1982).

Monopolar tungsten stimulation electrodes were positioned in the stratum radiatum of both slices. Stimulation consisted of monophasic square-wave pulses (0.1 msec duration). Extracellular population responses were recorded in the stratum pyramidale of the CA1 through glass micropipettes filled with 4M NaCl (5 Mohm resistance). Responses were amplified with a Grass P-15 pre-amplifier with cut-off frequencies set at 0.1 Hz and 50 kHz. Data were analysed as described elsewhere in this thesis.

Stimulation of the stratum radiatum produced a positive EPSP and superimposed negative population spike, recorded in the stratum pyramidale. Test pulse intensity was set to produce a sub-maximal population spike. Test pulses were applied once every 15 seconds throughout testing. Following 10-30 minutes of baseline recording, 4mM CaCl was introduced for 10 minutes. Testing was continued for 30 minutes after normal CaCl was returned. At the end of the 30 minutes post-Ca^{++} testing, a single 100 Hz, 1 sec train was applied, and response amplitude monitored for an additional 10-30 minutes.

RESULTS

Six slice pairs were tested. Only one adult slice demonstrated Ca^{++}-induced potentiation, and this was short-term only. The population spike recorded in this slice attained a peak potentiation of 200% which decayed to baseline within 20 minutes after normal Ca^{++} was returned to the medium. All other adult slices, and all immature slices demonstrated a long-lasting Ca^{++}-induced depression of the population

spike. Four of five adult slices demonstrated STP/LTP of the population spike following high frequency stimulation. One of five immature slices demonstrated LTP, and four of five demonstrated STP, following high frequency stimulation.

DISCUSSION

The results reported here are in marked contrast to those reported by Turner, et al. (1982), who reported 14 of 17 mature slices demonstrating Ca^{++}-induced LTP of the population spike lasting at least 3 hours. Since no Ca^{++}-induced LTP was demonstrated in either age group, no comparisons can be made. The frequency of LTP in mature and immature slices following high frequency stimulation corresponds well with previous reports of the development of LTP in the CA1 (Baudry, et al., 1981; Harris & Teyler, 1982).