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by

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Abstract

LONG-TERM CHOLINERGIC STIMULATION OF PONTINE NUCLEI:
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by

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A number of studies have shown that acute administration of cholinergic agonists into the brainstem produces some or all of the tonic and phasic aspects of paradoxical sleep (PS). While these studies support the hypothesis that alterations in activity of a discrete set of reticular neurons might trigger PS, no study has charted long-term alterations in PS induced by chronic infusions of cholinergic agonist-antagonists into the pons. In this study, an Alzet osmotic mini-pump is used to infuse either carbachol (0.5 ug/hr), scopolamine (9.0 ug/hr) or saline into the brainstem or fourth ventricle.

In Experiment 1, 51 male rats are implanted with chronic indwelling EEG and EMG electrodes and an L-shaped cannula aimed at various brainstem sites or the fourth ventricle. Following recovery from surgery, a 24 hr baseline sleep record is obtained. The next morning the Alzet mini-pump is implanted and nine consecutive 24 hr sleep records obtained (7 days drug + 2 days post-drug). Two weeks later a 24 hr post-experimental record is obtained. Within groups repeated measures ANOVAs show that midline infusions of carbachol into the area around the genu of

the VII nerve produce an 88% increase in PS. Midline infusions into other brainstem areas or the fourth ventricle do not alter PS. The PS increase occurs only during the night cycle and is due to an increase in PS frequency. Scopolamine, on the other hand, decreases PS by an average of 60% and the effect is not site-specific. The PS decrease occurs during the day cycle and it is due to a decrease in PS frequency and duration.

In Experiment 2, the period of carbachol infusion is increased to two weeks. Midline infusions into areas caudal to the genu of the VII nerve produce contradictory results in that PS is increased during the day cycle and decreased during the night cycle. It is suggested that the day cycle PS increase might be a PS rebound.

Experiment 3 tests the effects of such chronic infusions on retention of a learned response. Independent groups of rats are implanted with pumps containing either carbachol, scopolamine or saline. Subsequently the rats are given 50 consecutive trials in a shuttlebox and tested for retention 7 or 21 days later. At 7 days the scopolamine animals show significantly poor retention compared to carbachol or saline animals. At 21 days, however, all animals show good retention. It is suggested that scopolamine may have produced a retrieval failure at 7 days.

The results of this dissertation provide further evidence of the important role of the cholinergic system in PS and memory.

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Introduction

On the basis of behavioral and electrophysiological observations, sleep in most animals can be classed into two states, a slow wave (SWS) phase and a paradoxical sleep (PS) phase. Normally, SWS precedes PS and it is characterized by the presence of large amplitude slow waves (1-4 Hz) in the EEG and, relative to waking, reduced EMG activity. PS, on the other hand, is identified by the occurrence of (1) low voltage fast activity and rapid eye movements (Aserinsky and Kleitman, 1953), (2) loss of muscle tonicity (Jouvet and Michel, 1959), (3) monophasic waves in pons, lateral geniculate nucleus and occipital cortex (PGO waves)(Brooks and Bizzi, 1963; Farber, Marks and Roffwarg, 1980), (4) very regular activity in dorsal hippocampus (theta activity)(Vanderwolf, 1969; Winson, 1972), (5) increased activity of pyramidal tract neurons (Evarts, 1964), and brainstem neurons (Steriade and Hobson, 1976), and (6) activity of the vestibular pathway (Bizzi, Pompeiano and Somogyi, 1964). These physiological events may be classified as tonic or phasic, depending upon whether or not the event occurs throughout the PS episode (eg., EEG low voltage fast activity, EMG suppression, theta waves), or periodically (eg., rapid eye movements, PGO waves).

Of the two states, PS has aroused the interest of researchers primarily because of its contradictory nature, ie., behaviorally the subject appears to be asleep but electrophysiologically the pattern of neural activity is similar to waking. Additionally, there is apparently a need for PS since any loss of this phase of sleep is made

up at a later time (Dement, 1960). Moreover, in humans, vivid dreaming is associated with PS (Dement and Kleitman, 1957).

The intriguing nature of PS has spurred researchers to characterize the nature of the neuronal events giving rise to each of the tonic and phasic aspects of PS, and to determine the biological significance of this state of sleep. After considerable research it has been determined that PS originates from within the pontine reticular formation (Jouvet, 1962). However, it is not known which pontine center is responsible for initiating PS. Recently, there has been considerable speculation that the cholinoceptive cells of the gigantocellular tegmental (FTG) may represent the PS trigger (Hobson and McCarley, 1977), and acute pharmacological experiments seem to support this contention (Vivaldi etal., 1980). However, it is still uncertain whether this area is able to produce sustained alterations in PS in response to chronic pharmacological stimulation. This is particularly important, since before a neural center can be designated a PS trigger area it must demonstrate divergent responses to opposing neurotransmitter modulating agents, over a prolonged time period.

The purpose of this dissertation is to determine whether long-term infusions of cholinergic agents into regions postulated to play a critical role in PS produce prolonged alterations in PS. An additional goal of this dissertation is to assess the effects of such pharmacological manipulations on memory. This latter aspect of the study is designed to test the hypothesis that activation of neuronal pathways during PS provide an atmosphere conducive to information processing. Evidence supporting such a biological role for PS is

quite extensive (for review see Fishbein and Gutwein, 1981), and it will be presented later. First, however, it is necessary to outline the anatomical organization of the neurotransmitter systems involved in PS, and to delineate the neural centers postulated to play a critical role in sleep and the various tonic and phasic aspects of PS.

Anatomical organization

The serotonin system

Dahlstrom ad Fuxe (1964) first reported the existence of serotonin containing cell bodies within the brainstem. These areas labelled B1-B9 lie along the midline and extend from the caudal medulla (nucleus raphe pallidus or group B1) to the midbrain (nucleus raphe dorsalis or group B7).

The anterior portion of the raphe (groups B7 and B8) contains the greatest concentration of serotonin neurons and accounts for nearly 80% of forebrain 5- HT (Azmitia, 1978). Detailed mapping utilizing a variety of methods such as histofluorescence (Dahlstrom and Fuxe, 1965), and autoradiography (Azmitia and Segal, 1978; Bobillier etal., 1975) have shown that while these groups project to the brainstem, cerebellum and spinal cord, the majority of the fibers ascend to innervate the cortex, hippocampus, septum, hypothalamus, thalamus and amygdala. The pontine- medullary raphe nuclei (groups B1-B6), on the other hand, project mainly to the brainstem, cerebellum and spinal cord.

Witnin the brainstem itself, raphe dorsalis projections to the locus coeruleus (LC) have been demonstrated in the rat Morgane and

Jacobs, 1979; Leger and Descarries, 1978), and cat (Sakai etal., 1977). The LC, in turn, has been shown to innervate the dorsal raphe (Loizou, 1969; Roizen and Jacobowitz, 1976). Other areas with afferents to the dorsal aphe include the hippocampus, lateral habenular nuclei, pontine reticular formation, spinal cord and the median raphe (Azmitia, 1978).

In summary, the serotonin containing raphe nuclei lie within the core of the reticular formation and extensively innervate the entire brain and spinal cord. In turn, these nuclei receive both noradrenergic and cholinergic afferents (Lewis and Shute, 1967; Chu and Bloom, 1974; Saavedra etal., 1976).

The norepinephrine system

Characterization of the ascending and descending norepinephrine (NE) system has been greatly facilitated by the development of several anatomical methods, such as fluorescence histochemistry (Falck etal., 1962), immunofluorescence of catecholamine systnesizing syzymes (Hokfelt etal., 1975; Swanson and Hartman, 1975), autoradiographic tracing method (Cowan etal., 1972), and horseradish peroxidase (HRP) method (Lavail and Lavail, 1972).

Dahlstrom and Fuxe (1964) have identified several discrete NE-containing regions within the brainstem. These cell groups, labelled A1-A7, extend from the ventrolateral aspects of the medullary tegmentum (A1 and A3) to the dorsolateral parts of the pontine brainstem (A7). Ascending and descending NE axons originating from these regions provide extensive innervation of the brain and spinal cord (Lindvall and Bjorklund, 1978). To summarize, the dorsal

tegmental bundle, originating exclusively in the locus coeruleus (A1)(Maeda etal., 1973; Lindvall etal., 1974), provides widespread innervation of the cortex, hippocampus, thalamus, hypothalamus, amygdala and septum (Ungerstedt, 1971; Lidbrink and Jonsson, 1974; Lindvall eta., 1974). A second major system, the ventral tegmental bundle, has its roots in cell groups A1, A2, A5, and A7 (Ungerstedt, 1971), and projects diffusely to the brainstem, hypothalamus, basal forebrain and spinal cord (Lindvall and Bjorklund, 1978).

In comparision to the other brainstem NE cell groups, the LC is the largest and is composed virtually entirely of NE (Dahlstrom and Fuxe, 1964). The dorsal tegmental bundle originates in the rostral portions of the LC and innervates virtually the entire brain. Some of the structures receiving efferents from this fiber system are the superior and inferior colliculus, septum, hippocampus, amygdala, hypothalamus, and raphe nuclei (Anderson etal., 1977; Loizou, 1969; Chu and Bloom, 1974; Lindvall and Bjorklund, 1978; Jones and Moore, 1977; Ungerstedt, 1971; Kobayashi etal., 1974; Maeda and Shimizu, 1972; Pickel etal., 1974). Fibers emanating from the medial portions of the LC project to the cerebellum (Olson and Fuxe, 1971; Ungerstedt, 1971; Segal etal., 1973; Bloom etal., 1974; Pickel etal., 1973), while a descending pathway, the bulbo-spinal pathway (Loizou, 1969; Maeda etal., 1973; Sachs etal., 1973; Dahlstrom and Fuxe, 1965), terminates within the lumbar and thoracic segments of the spinal cord (Kuypers and Maisky, 1975).

Other areas receiving efferents from the LC include the nucleus of the solitary tract (Loizou, 1969), and the lateral reticular areas of

pons and midbrain (Morgane and Jacobs, 1979).

To summarize, NE is well represented in the brainstem and major ascending and descending fiber systems originate in the brainstem reticular formation.

The acetylcholine system

The status of acetylcholine as a neurotransmitter has been known for some time. However, lack of accurate mapping procedures comparable to those used to localize serotonin and NE has hindered progress in determining the organization of this system. The recent development of a new autoradiorgraphic procedure which locates muscarinic receptors (Wamsley etal., 1981) promises to change this, and data from this and other histological studies (Kimura and McGeer, 1981) is only now providing information regarding the significance of this neurotransmitter in sleep and other behaviors. Since a variety of histochemical methods have been used to map the acetylcholine (Ach) system, a summary of these techniques is presented below.

One method used widely (Lewis and Shute, 1978; Palkovits and Jacobowitz, 1974) involves detecting acetylcholinesterase (AchE), the enzyme which metabolizes Ach. This method, developed by Koelle and Friedenwald (1949), however, is suspect principally since it provides only an indirect method of localizing Ach neurons. Another limitation of this procedure is that AchE is found in places where there is no Ach. A more specific histochemical marker of Ach pathways is choline acetyltransferase (CAT), a synthesizing enzyme of acetylcholine (Bull etal., 1963). Other procedures include locating areas which display a high-affinity choline uptake system (Sorimachi and Kataoka, 1974), and

determining the presence of specific receptors for Ach (Wamsley etal., 1981). Comparision of the data from each of these methods shows a high degree of similarity (Lewis and Shute, 1978), particularly between the CAT and choline uptake methods.

Two major ascending cholinergic pathways arise from the midbrain reticular formation (Lewis and Shute, 1967; Shute and Lewis, 1963, 1967; Lewis and Shute, 1978). The dorsal tegmental pathway originates within the dorsal tegmental region and projects widely to the optic tectum and thalamus. The ventral tegmental pathway has its roots within the ventral tegmentum and projects to the cortex, thalamus, lateral hypothalamus and basal telencephalic areas. Both fiber systems reeive input from brainstem nuclei and affect the whole brain. However, unlike the monoaminergic system, which projects without interruption to rostral regions, the ascending cholinergic fibers synapse with other cholinergic cells, which then innervate more distal regions. Via such second and third order projections, the amygdala, septum, hippocampus, dentate gyrus and cortex are innervated.

Within the brainstem itself, the solitary tract nucleus stains heavily for AchE and also has a high CAT activity (Kobayashi etal., 1975). Other areas displaying AchE activity include the LC, dorsal raphe, the FTG and the pontis caudalis nucleus (Palkovits and Jacobowitz, 1974; Bieger and Harley, 1982). Muscarinic receptors have also been found in these areas as well as others such as tne nucleus of the facial nerve and the medial vestibular nucleus (Wamsley etal., 1981). Of particular importance to this dissertation is the finding of muscarinic receptors within the FTG, nucleus reticularis pontis

caudalis and on the floor of the fourth ventricle (Wamsley etal.,1981).

To summarize, the cholinergic fibers eminating from the brainstem comprise the ascending cholinergic system (Shute and Lewis, 1963, 1967). The possibility that this system corresponds to the ascending reticular activating system (Moruzzi, 1972) is suggested by data from studies in which reticular stimulation has been shown to release Ach in the cortex (Szerb, 1967), and produce arousal (Celesia and Jasper, 1966).

Sleep and PS as active processes

Early researchers believed that sleep was a passive phenomenon, due to cessation of afferent activiy (see Moruzzi, 1972). Confirmation of this hypothesis was provided by Bremer (1937) who showed that cats with transections at the caudal end of the medulla (encephale isole) exhibited normal sleep-wake cycle whereas animals with mid-collicular transection (cerveau isole) displayed a permanently synchronized EEG. Bremer reasoned that a cerveau isole animals slept because the transection blocked all sensory input to the brain.

Such a view prevailed until Moruzzi and Magoun (1949) applied electric shocks to the reticular formation of encephale isole or chloralose anesthetized cats and produced immediate and long lasting arousal. These findings suggested an alternative explanation for the somnolence of cerveau isole cats; perhaps the reticular formation, instead of the sensory impulses, was responsible for arousal. Confirming evidence was provided in a series of experiments in which only the ablation of the reticular core resulted in a permanently

synchronized EEG (Lindsley etal., 1950).

Subsequently, Moruzzi and his group conducted a series of experiments designed to isolate the region of the reticular formation involved in waking. They noted that if the cuts were made just anterior to the entrance of the fifth nerve in the pons (mid-pontine pretrigeminal transection) the animals manifested insomnia (Batini etal., 1959). Such a preparation had the same sensory input as the cerveau isole animals, but unlike the somnolence of the cerveau isole, the mid-pontine pretrigeminal animals had insomnia. This finding suggested that a restricted region of the pontine reticular formation, just anterior to the fifth nerve, played a critical role in wakefulness.

A final set-back for Bremer's view that sleep resulted from a passive reaction to reduced sensory input was provided in an experiment by Batsel (1964) who showed that in a cerveau isole preparation, if the cuts were made in stages then the animals would display wakefulness followed eventually by a sleep-wake cycle. In fact, Villablanca (1962, 1965) has shown that in a high cerveau isole (Pre- collicular transection) wakefulness is seen 7-10 days after the section.

Moruzzi (1972), in summarizing these findings, suggests that the brainstem reticular core is responsible for maintaining arousal, but there are centers elsewhere in the brain which can produce sleep and wakefulness. The existence of another waking center located in the posterior hypothalamus is suggested by studies in which lesions of the medial and lateral portions of the posterior hypothalamus produce

somnolence (Nauta, 1946; Naquet, etal., 1966; Swett and Hobson, 1968). Interpretation of these findings is difficult particularly since the lesions may have induced sleep by destroying the ascending reticular impulses coursing through the medial forebrain bundle.

The status of a forebrain sleep center is much stronger. Nauta (1946) initially observed that lesions of the pre-optic basal forebrain area produced a rat with insomnia. McGinty and Sterman (1968) have confirmed these findings and shown that PS appears 4-6 weeks later only after SWS had reached 15% of total time. Electrical stimulation of this region has been shown to produce both EEG as well as behavioral signs of sleep (Sterman and Clemente, 1962). In fact, sleep can be induced by a conditioning procedure in which a tone presented with electrical stimulation begins to initiate sleep in the absence of any stimulation (Clement, Sterman and Wyrwicka, 1963). Moreover, Roberts and Robinson (1969) have shown that diathermic warming of this region produces sleep.

These findings suggest that structures located in the basal forebrain area provide an inhibitory influence on ascending reticular activating structures. Indeed, Bremer (1970) has shown that stimulation of the basal forebrain inhibits neurons within the midbrain reticular formation.

The basal forebrain area is not the only region with antagonistic influences on the ascending reticular system. The hint that structures in the lower brainstem dampen the reticular system came from studies in which mid- pontine pre-trigeminal transections produced animals with insomnia (Batini etal., 1959); encephale isole

animals, on the other hand, display normal sleep- wake cycle. Further evidence for a medullary sleep center is provided from studies (Magni etal., 1959) in which arousal is obtained by thiopental injections into the intravertebral artery serving the caudal brainstem); sleep results from injections into the carotid artery. Moreover, cooling of the medullary floor of the fourth ventricle in encephale isole preparations during synchronized sleep or drowsiness produces arousal, while cooling of the ventricle at the pontine level produces sleep (Berluchi etal., 1964, 1965). these findings have been confirmed in the intact cat (Naquet etal., 1966).

The medullary sleep center appears to be located in the region of the solitary tract nucleus, since stimulation of this area produces EEG synchrony which outlasts the stimulation (Magnes etal., 1961). Further support is provided by Bonvallet and Allen (1963), who have shown that lesions of the solitary tract nucleus considerably enhance and prolong the phasic arousal produced by reticular stimulation. Additionally, 5-HT application to the nucleus induces SWS (Key and Mehta, 1977), and solitary nucleus cells undergo significant increases in discharge rates during SWS (Eguchi and Satoh, 1979). Finally interactions between the nucleus and the reticular formation have been demonstrated (Bronzino, 1972).

To summarize, it is clear that the original view of sleep being a passive phenomena is no longer tenable. In its place, a theory which proposes that sleep and wakefulness are under the control of specific

brain regions appears to be more viable (Jouvet, 1972). Such a theory would also account for PS, particularly since it would suggest that at regular periods during sleep discrete neural centers are able to lift the inhibitory influence on the ascending reticular activating system and allow ascending impulses to predominate for a few minutes (Jouvet, 1972). It is important now to delineate those nuclei postulated to be involved in generating and maintaining PS.

The role of the pontine nuclei in sleep: A review

Very soon after the discovery of PS, Jouvet (1962) determine that PS originated from within the pontine brainstem. For expample, he noted that in a chronic pontile cat (transections at the rostral end of the pons), the EEG activity in front of the transection is similar to a chronic cerveau isole preparation, that is continuous mixing of spindles and slow waves regardless of state of wakefulness. However, behind the cut the animal's sleep, which can be identified by the occurrence of spindles at the pontine level, is interrupted often by episodes of PS. These episodes are characterized by complete disappearance of muscle activity (loss of decerebrate rigidity). Jouvet notes that this is indeed PS since the duration of atonia is similar to intact animals, and each episode is marked by irregular respiration, relaxed nictating membrane and a 25% increase in auditory arousal threshold compared to awake. Moreover, he has shown that if the cuts are made at the caudal end of the pons, PS is not seen even though SWS can be observed 40% of the time. Further evidence in support of a pontine PS generator is provided by studies in which lesions of the pontine reticular formation produces no PS but SWS and

wakefulness remain intact (Jouvet, 1962). He has also shown that cerebellectomy or decortication do not alter PS (Jouvet, 1962).

Since Jouvet's pioneering studies, researchers have identified several nuclei within the pontine brainstem which may contribute towards generating and regulating the various tonic and phasic aspects of PS. Moreover the neurotransmitters associated with these areas have also been implicated in the PS process.

The role of the raphe nuclei and serotonin in sleep and PS

Jouvet (1972) has suggested that the integrity of the serotonin containing raphe nuclei is essential for sleep, mainly SWS, and that these neurons are part of a PS priming mechanism with links to the PS generator. The evidence supporting such a role comes from a variety of ablation and pharmacological experiments in which it has been demonstrated that SWS and PS are influenced by brain 5-HT levels.

Increase in availability of 5-HT

Application of 5-HT in the region of the area postrema, which is close to some 5-HT neurons, or in the ventricles, induces cortical synchronization in the cat (Bronzino etal., 1972; Koella and Czicman, 1966). Furthermore, injections of 5-HT into the arteries supplying the area postrema produces cortical synchronization, while application of NE increases cortical desynchronization (Roth etal., 1970).

Elevation of 5-HT by adminstration of its biosynthetic precursor, tryptophan, increases SWS in man and decreases the lateny to sleep onset (Wyatt, 1972; Hartmann, 1977). Moreover, a tryptophan free diet reduces total sleep time and the frequency of PS (Hartmann, 1968). However, Lanoir etal., (1981) report no changes in waking, SWS or PS

in rats maintained on a tryptophan free diet, inspite of a 50% reduction in 5-HT.

5-HT can also be increased by application of its precursor, 5-HTP, which readily crosses the blood-brain barrier. Delorme (1966) reports that in cats, low doses (1-5 mg/kg) of 5-HTP produces synchronization and a slight increase in PS. However, in humans, 5-HTP does not affect SWS but does increase PS (Wyatt, 1972). Furthermore, Ursin (1976) reports that in cats injections of 5- HTP or tryptophan does not influence SWS.

Low frequency electrical stimulation of the dorsal raphe, in rats, has been shown to induce behavioral and EEG sleep (Kostowksi etal., 1969; Gumulka etal., 1971). However, no such sleep inducing effects have been noted either in rabbit (Polc and Monnier, 1970) or cat (Siegel and Brownstein, 1975; Jacobs etal., 1973; Bronzino etal., 1976).

With respect to circadian alteration of 5-HT, elevated levels of 5-HIAA (5-HT metabolite) during SWS as compared to wakefulness has been reported in cats (Buckingham and Radulovacki, 1975), and man (Wyatt etal., 1974). Also decreased brain 5-HT has been noted in cats sacrificed during SWS (Sinha etal., 1973).

Reduced availability of 5-HT

Raphe lesions have been found to be effective in reducing brain 5-HT while leaving NE levels unchanged (Jouvet, 1972). Such lesions produce an immediate behavioral and EEG arousal lasting for 3-4 days. Subsequently, SWS reappears but does not exceed 10% of total time (Jouvet, 1969). Furthermore, partial raphe lesions do not produce

total insomnia, and PS is seen only if SWS exceeds 15% of total time. Injections of low doses of 5-HTP do not alter the insomnia in lesioned animals. High doses of the precursor, on the other hand, induce cortical synchronization followed by waking behavior (Renault, 1967; Pujol etal., 1971). These findings have been confirmed in the rat (Kostowski etal., 1968).

More discrete lesions of the raphe have been found to produce a differential effect on PS. For example, while total raphe lesions completely suppress SWS and PS, anterior raphe lesions (nucleus raphe dorsalis and centralis) induce a state of permanent arousal for 2-3 days during which PS occurs 5-10% of the time; the PS occurs, in fact in the absence of any SWS (narcolepsy) (Jouvet, 1972). Caudal raphe lesions (raphe magnus and pontis), on the other hand, produce an almost total disappearance of PS even though SWS is decreased to only 40% of control values. On the basis of these findings Jouvet (1972) suggests that the anterior raphe neurons contribute towards SWS while caudal raphe neurons promote PS.

Another method used to reduce 5-HT is the injections of para-chlorophenylalanine (PCPA) (Koe and Weissman, 1966; Gall etal., 1970). This drug acts by irreversibly binding to tryptophan hydroxylase, the rate limiting enzyme in the synthesis of 5-HT, and 5-HT recovery does not occur until new enzyme is synthesized (Jequir etal., 1967). A single intraperitoneal dose of PCPA lowers brain 5-HT content to less than 20% within 3 days, and complete recovery does not occur for about two weeks (Weissman, 1973).

The effects of PCPA on the sleep-wake cycle have been studied most

extensively in the cat (Jouvet, 1972), where a single injection of PCPA produces no effects until 18-24 hours after injection. Following this initial period, a gradual decrease in sleep begins to occur until almost total insomnia is seen 30-40 hours later. Subsequent to this, sleep begins to reappear and normal sleep-wake patterns are seen 200 hours following the PCPA injection. Correlative evidence linking 5-HT to sleep is provided by studies which show that the PCPA induced decrease in sleep closely coincides with a fall in 5-HT levels (Koella etal., 1968; Pujol etal., 1971; Bobillier etal., 1973).

The PCPA induced decrease in 5-HT can be reversed almost immediately by administration of 5-HT (Hoyland etal., 1970), and this recovery is correlated with a rise in sleep (Mouret etal., 1967; Koella etal., 1968; Jouvet, 1972; Pujol etal., 1971).

Similar findings have been reported in the rat (Torda, 1967; Mouret etal., 1968), rabbit (Florio etal., 1968), and monkey (Weitzman etal., 1968).

Reserpine, which reduces 5-HT by disrupting storage sites, has also been used to assess the role of 5-HT in sleep. Any findings regarding this drug, however, must be viewed with caution, since the effects of reserpine are not confined to 5-HT; it disrupts catecholamine vesicles also (Sulser and Bass, 1968). In the cat, reserpine induces sedation where EEG activity is quite different from sleep or relaxed wakefulness. The first signs of SWS do not appear until 6-8 hours after injection, and PS is not seen for 1 or 2 days (Matsumoto and Jouvet, 1964). During this period of sedation, administration of 5-HTP produces cortical synchrony immediately

(Jouvet, 1972), while L- Dopa (a catecholamine precursor) hastens the onset of waking and PS (Matsumoto and Jouvet, 1964). This evidence has led Jouvet (1972) to suggest that SWS is under the control 5-HT while PS and waking are regulated by NE.

While reserpine, PCPA and raphe lesions produce widespread disruption of the 5-HT syste, and raphe lesions destroy ascending and descending pathways, other techniques have been used which affect discrete populations of 5-HT neurons. For instance, 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-DHT administered intraventricularly or intracisternally degenerate 5-HT neurons and have a rather selective depleting action on central serotonin levels (Baumgarten etal., 1977). Of the two, 5,7-DHT is used more frequently, since it does not possess the toxic properties of 5,6-DHT (Baumgarten etal., 1973), and when used in conjunction with monoamine oxidase inhibitors, 5,7-DHT attenuates 5-HT levels without altering catecholamine levels (Baumgarten etal., 1977). Froment etal., (1974) have reported that intraventricular injections of 5,6-DHT in cats produce a small decrease in SWS. However, they also show that when the drug is injected into the raphe, no effect is seen. Intraventricular injections of 5,7-DHT in rats, while depressing serotonin by 78% do not decrease SWS (Ross, Trulson and Jacobs, 1976). However, Kiianma and Fuxe (1977) report that 5,7-DHT in rats does significantly reduce SWS and increase wakefulness.

While these studies support Jouvet's contention that 5-HT and the raphe play a critical role in inducing SWS, there is mounting evidence which contradicts this position. For example, it has already been

noted that single injections of PCPA induce insomnia. However, chronic administration of the drug while depressing 5-HT levels by 90-95% do not produce any loss of SWS or PS (Rechtschaffen etal., 1973; Dement etal., 1972; Dement, Henriksen etal., 1973; Cohen etal., 1970). Moreover, in raphe-lesioned animals monitored over a long period of time, sleep has been shown to return to normal levels (Mouret and Coindet, 1980; Juvanez, 1980; Morgane and Stern, 1974; Bouhuys and Van Den Hoofdakken, 1977). Furthermore, Adrien etal., (1977) have shown that newborn rats with midbrain raphe lesions do not show a decrease in sleep three weeks post lesion even though 5-HT levels are 5-10% of control.

A possible reason for the transient insomnia seen in raphe lesioned or PCPA/reserpine treated animals is that serotonin depletion produces hyperactivity and hyper-excitability (Dement etal., 1973; Jacobs etal., 1974, 1975; fibiger and Campbell, 1971; Kostowski etal., 1968; Lorens etal., 1971; srebro and Lorens, 1975). As such, Dement etal., (1973) suggest that the insomnia is due to hyper-responsivity to both externally and internally generated sensory events; the latter would be PGO spikes, which in normal animals are confined to PS, but occur during waking when 5-HT is depleted. When viewed in this context, it is suggested (Ursin, 1976; Jacobs and Jones, 1978; Trulson and Jacobs, 1979) that the raphe or 5-HT may modulate, rather than initiate, the onset of sleep by exerting a general deactivating effect on the waking state.

Moreover, the firing pattern of raphe neurons is not in accord with Jouvet's hypothesis that the raphe is actively involved in

initiating SWS, since these neurons actually exhibit a 50% decline in discharge rate during SWS when compared to waking (McGinty and Harper, 1976; Sheu, Nelson and Bloom, 1974; Trulson and Jacobs, 1979). Additionally, except in the rat, stimulation of the raphe does not produce sleep.

In view of this contradictory evidence it would appear that Jouvet's hypothesis needs to be revised in that the raphe may modulate rather than initiate sleep. Considering the evidence from various studies, it seems that sleep may be initiated by tonic inhibitory impulses arising from the basal hypothalamus and the solitary tract nuclei, and that these nuclei may then interact with the raphe in promoting a physiological state conducive to sleep.

With respect to the role of the raphe in priming PS, the evidence is quite strong that depletion of 5-HT beyond a certain threshold prevents PS from occuring and that these priming neurons are located within the caudal raphe. One consistent finding to emerge from the raphe studies is that the raphe plays a very important role in regulating one phasic aspect of PS, viz., PGO.

The raphe, 5-HT and PGO

PGO are slow monophasic waves appearing either as isolated waves (23%) or in groups of 3-4 (60%) in the pons, lateral geniculate body and occipital cortex (Brooks and Bizzi, 1963; Mouret etal., 1963). While the cat is the only animal from which the PGO have been recorded, and extensive studies on the PGO have been done using this animal, recently there has been some evidence to indicate that a homologue of the PGO may be present in the rat (Farber, Marks,

Roffwarg, 1980).

PGO spikes begin to occur 30-60 secs prior to PS onset, and during PS itself the frequency of PGO increases from 10 per minute to 60 per minute (Jouvet, 1972). Normally the PGO are confind to PS but on rare occassions they may occur outside of PS (Jouvet, 1972). Other interesting characteristics of PGO are that the number of PGO discharges are constant from one PS episode to the next (Buguet, 1969), the total daily number of PGO is constant (approximately 13000) (Jouvet, 1972), and during the rebound which follows PS deprivation, PGO occur during PS and SWS at higher frequency until the PGO debt is almost totally repaid (Dement etal., 1970).

Administration of reserpine (0.5-1 mg/kg) triggers the release of PGO into waking after 60-90 minutes following the injection (Delorme etal., 1965; Brooks and Gershon, 1971). These reserpine PGO are similar to normal PS PGO in that they are accompanied by discharge in external rectus muscle and rapid eye movement (Jouvet, 1972). Other treatments which release PGO into waking include PCPA (Delorme etal., 1966; Dement etal., 1970), p-chloromethamphatamine (Delorme etal., 1966), and raphe lesions (Jouvet, 1972). The fact that these treatments also decrease 5-HT suggests that 5-HT exerts an inhibitory control on PGO. This is confirmed from studies in which replenishment of 5-HT immediately suppresses PGO (Jouvet, 1972).

Considering the evidence, the most parsimonious hypothesis regarding PGO is that tonically active 5-HT neurons within the raphe play an inhibitory role in PGO generation, and that when these neurons decrease their firing during PS or there is 5-HT depeltion then PGO

occur (Brooks and Gershon, 1971; Simon, Gershon and Brooks, 1973). The viability of this hypothesis is suggested from single unit studies which show complete cessation of activity of the dorsal raphe neurons just before and in temporal contiguity with PGO activity (McGinty and Harper, 1976; Sheu, Nelson and Bloom, 1974; Trulson and Jacobs, 1979). Moreover, Jacobs etal., (1973) have demonstrated that electrical stimulation of the raphe suppresses PGO during PS. However, the raphe represents only one center modulating the PGO since other pontine nuclei have also been implicated in the PGO process (Jouvet, 1972; McCarley, Nelson and Hobson, 1978; Valleala etal., 1979).

Summary

The role of the serotonergic raphe neurons in sleep may be as follows: (1) These neurons may interact with the solitary tract nucleus and the cells in the basal hypothalamus in promoting a physiological state conducive to sleep; in this regard the raphe may serve to modulate, rather than initiate, the onset of sleep. (2) The caudal raphe may be responsible for priming PS. (3) The raphe exerts an inhibitory control in regulating the occurrence of PGO.

The role of norepinephrine and the locus coeruleus in waking and PS

The norepinephrine (NE) conatining locus coeruleus (LC) neurons are regarded as playing a major role in both waking and PS (Jouvet, 1972; Stern and Morgane, 1974). Such a role is possible considering that the LC, with its widespread projections, innervates virtually the entire CNS. The experimental approaches taken to investigate the involvement of this system in waking and PS include altering central NE levels pharmacologically or lesiong the LC, and by monitoring the

discharge patterns of LC neurons in conjunction with the sleep/wake cycle.

Increase in availability of NE

Since NE fails to cross the blood-brain barrier, central NE levels can be increased by administering its precursor, L-Dopa. Evidence from cats (Delorme, 1966; Jones, 1972) and rabbits (Monnier and Tissot, 1958) indicate that L-Dopa induces a state of arousal for several hours. Moreover, the arousal is enhanced by peripheral inhibition of dopa-decarboxylase (Bartholini etal., 1967). The most striking effects of L-Dopa is seen in reserpine treated animals where it reverses the sedation and results in normal waking (Jouvet, 1978; Brooks and Gershon, 1977).

Amphetamine increases the availability of NE at synapses by a variety of methods including blockade of NE reuptake and MAO inhibition (Gorelick etal., 1978). In kittens (Shimizu and Himwich, 1968) and cats (Jewett and Norton, 1966), amphetamine induces EEG and behavioral arousal, and suppresses SWS and PS. Perhaps the arousal effects are mediated in the brainstem reticular formation since destruction of the reticular formation, or intercollicular transection suppresses the effect (Hiebel etal., 1954). Moreover, if amphetamine is administered intravertebrally and the basilar artery tied at the midpontine level, there is no arousal (Van Meter and Ayala, 1961).

NE availability can also be increased by inhibition of the catabolizing enzymes. The MAO inhibitor nialamide produces arousal in rabbit and cat (Mouret etal., 1968). However, nialamide also suppresses PS (Delorme, 1966). Prenylcypromine and pheniprazine

increase waking but decrease PS, and when used in conjunction with L-Dopa, an intense arousal is seen (Jones, 1972). Pargyline, on the other hand, reduces waking but when used with L-Dopa the somnolence is reversed (Jones, 1972). Other investigators have also found that MAO inhibitors generally suppress PS and produce no subsequent PS rebound (Gillin etal., 1978; Stern and Morgane, 1974).

The administration of catechol-o-methyl transferase inhibitor tropolone, in cats, produces arousal for several hours followed by rebound of PS and SWS (Jouvet, 1978). L-Dopa plus tropolone induces intense arousal for several hours followed by hallucinatory episodes (Jones, 1972).

Pharmacological manipulations affecting pre- and post- synaptic receptors can also influence NE activity. In cats, the alpha-adrenergic blockers phenoxybenzamine and dibenamine suppress PS and increase SWS (Matsumoto and Watanabe, 1967). However, Hartmann etal., (1973) tried phenoxybenzamine at high doses (40 mg/kg) and found it to increase PS and decrease waking. This has been confirmed in the rat (Hartmann and Zwillig, 1976), and humans (Oswald etal., 1975). Moreover, another alpha-adrenergic blocker, phentolamine, also induces PS in cats (Putkonen and Leppavouri, 1977). The beta-adrenergic receptors apparently do not influence the sleep-wake cycle since blockade with nethalide (Matsumoto and Watanabe, 1967), propranolol (Hartmann etal., 1973), pimozide (Sagales etal., 1975), or chlorpromazine (Sagales etal., 1969) do not alter sleep. However, there is evidence tht chlorpromazine's effect may be dose dependent, since at low doses it increases PS while at high doses a PS decrease

is seen in rats and humans (Kafi and Gaillard, 1978; Gaillard and Kafi, 1979). One alpha-blocker with such dual action is yohimbine, which at low doses (100 ug/kg) increases PS while at higher doses (3000 ug/kg) it decreases PS (Kafi and Gaillard, 1980).

The administration of clonidine, an alpha receptor agonist, leads to sedation in normal and PCPA treated insominiac cats (Holman etal., 1971). In man it suppresses PS (Autret etal., 1976). The PS decrease can be antagonized by phentolamine (Putkonen and Leppavouri, 1977) and yohimbine (Putkonen etal., 1977).

In summary, there is evidence to suggest that an increase in NE leads to increase behavioral and EEG waking. There is however, considerable ambiguity regarding the effects of increase NE on PS. The results from MAO inhibitors and receptor blocking studies would suggest that a PS decrease is associated with an increase in NE. This directly contradicts Jouvet's hypothesis that PS is directly correlated with NE activity. The dose dependent effects obtained by Kafi and Gaillard (1980), however, indicate that results from receptor blocking studies should be viewed with caution, particularly since the data indicate that at low doses, alpha blockers act on pre-synaptic receptors and facilitate release of the transmitter (Starke etal., 1977; Langer, 1977); at higher doses, the drugs act on post-synaptic receptors (Kafi and Gaillard, 1980). The results confirm this since at low doses yohimbine and chlorpromazine elevate PS while at higher doses a PS decrease is seen.

Decrease in NE availability

Central NE can be decreased by inhibiting tyrosine hydroxylase the

rate limiting enzyme in the synthesis of catecholamine. Alpha-methyl-p-tyrosine (AMPT) is most widely used to inhibit tyrosine hydroxylase. AMPT induces sedation which can be counteracted by L-Dopa (Weissman and Koe, 1965), and it decreases waking even in amphetamine or raphe lesioned animals (Hanson, 1967; Jouvet, 1972). In monkeys (Weitzman etal., 1969) and rats (Torda, 1968) implanted with AMPT crystals, a decrease in PS is observed. In cats, Iskander and Kaelbling (1970) found decreases in PS during AMPT treatment. However, after the last injection PS increased dramatically.

Not everyone, however, has found that AMPT decreases PS. For example, in cats (King and Jewett, 1971; Stern and Morgane, 1973; Henriksen and Dement, 1972), and rats (Hartmann etal., 1971), AMPT has been found to increase PS. However, Kafi etal., (1977) suggest that these findings are due to dose dependent effects since they found that at low doses (75-100 mg/kg) AMPT increased PS while high doses (greater than 150 mg/kg) decreased it.

NE can also be decreased by inhibition of dopamine-beta hydroxylase. In cats, disulfiram significantly decreases waking and PS (Dusan-Peyrethon and Froment, 1968). Fusaric acid decreases PS also without having any effect on waking (Satoh and Tanaka, 1973).

Reserpine reduces NE levels by disrupting storage vesicles. Acute administration of reserpine (Hartmann and Cravens , 1973; Coulter etal., 1973) and AMPT (Hartmann etal., 1971) while decreasing NE increase PS. In fact chronic reserpine treatment, but not treatment with AMPT, increases PS (Gillin etal., 1978).

More selective changes in central NE can be achieved by lesioning

discrete NE pathways using 6-hydroxydopamine (6-OHDA) (Breese and Cooper, 1977). Intraventricular injections of 6- OHDA in rats produce a small decrease in waking but increase PS (Hartmann etal., 1971). Matsuyama etal., (1973) found increases in SWS followed by increases in waking. In this study PS decreased initially but then quickly returned to baseline levels. When 6-OHDA is injected into the dorso-lateral part of the pontine tegmentum (LC area), PS declines in the first four days and then PS is totally suppressed during subsequent days (Buguet etal., 1970). Cats killed on the 8th day show significant reductions in 5-HT and NE. Zolovic etal., (1973) found similar results with such injections.

Before 6-OHDA was discovered, central NE levels were disrupted by electrical ablation of the LC (Rousel etal., 1967). Drawing on the evidence from these initial lesion studies and considering the widespread projections of the LC, Jouvet (1972) suggested that the executive mechanisms for PS were located in the LC. These observations, however, were made from studies in which large lesions of the dorsolateral pontine tegmentum ie., the LC and significant protions of regions ventral to it, depleted central NE and abolished all signs of PS (Jouvet, 1972). Since then, more discrete lesions, involving only the LC, have shown that these lesions do not alter cortical desynchrony, one of the primary signs of PS, in cat (Jones etal., 1977) and rat (Robinson etal., 1977; Monmour and Delacour, 1978). Moreover, electrical stimulation of the LC has not been shown to induce cortical desynchronization (Robinson etal., 1977; Olpe etal., 1980).

In summary, there is considerable controversy regarding the role of NE and the LC in PS. On the one hand, data from AMPT studies indicate that PS is inversely related to NE availability, while other studies, using AMPT also, show a direct correlation between decline in NE and PS. Perhaps the most damaging evidence against the role of the LC as a primary PS generator comes from lesion studies. However, the lesion studies show that while the LC may not be involved in cortical desynchrony, it may play a very important role in PGO spike generation, and the muscle atonia which persists throughout PS.

The LC and muscle atonia

The evidence that the LC may be involved in activating the muscle atonia which accompanies PS, comes from studies in which cats with bilateral lesions of the caudal aspects of the LC, upon entering PS, suddenly become mobile and appear as if they were acting out dreams (Henley and Morrison, 1974; Jouvet, 1979).

Sakai (1980) and others (Chase, 1980) have speculated as to the pathway of the motor system inhibition during PS. They suggest that the atonia is due to the activation of the descending inhibitory pathways of Magoun and Rhines (1946), which then produce hyperpolarization of spinal alpha motor neurons (Glenn etal., 1978; Morales and Chase, 1980; Chase, 1980). Evidence from single-unit studies (Kanamori etal., 1980; Sakai etal., 1979) suggest that the source of the descending inhibition is the magnocellularis nucleus, which in turn may be activated by the LC (Sakai, 1980). Anatomical connections between the two nuclei have been demonstrated (Sakai etal., 1979; Tohyama etal., 1979). Recently there has been some

evidence that a cholinergic input into the LC may also be involved, since infusions of carbachol, a muscarinic receptor antagonist, into the LC (Van Dongen, 1980), and regions ventral to it (Mitler and Dement, 1974) triggers atonia and cataplectic behavior.

The LC and PGO

The LC may be involved in inhibiting PGO spikes because LC lesions result in the occurrence of PGO spikes during waking and SWS (Roussel, 1967; Buguet, 1969; Jalfre etal., 1974; Jones etal., 1977). Moreover, MAO inhibitors depress PGO (Jones, 1972), and tropolone plus L-Dopa release PGO into waking and SWS (Jones, 1972). The latter treatment also increases the frequency of PGO in PS. Amphetamine suppresses PCPA induced PGO (Jacobs etal., 1972).

There is, however, some controversy regarding the inhibitory action of NE and the LC on PGO, particularly because treatments which decrease NE, such as AMPT and disulfiram, do not increase PGO, as would be expected under the above hypothesis, but instead decrease PGO (Pyrethon-Dusan, 1968). Further, alpha- receptor blockers do not induce PGO into waking or increase frequency of PCPA induced PGO activity (Jacobs etal., 1972).

The discharge pattern of LC neurons

Evidence derived from single-unit studies indicate that LC neurons exhibit changes in firing patterns during PS. For example, Chu and Bloom (1973, 1974), and Hobson etal., (1976) have observed two types of neurons in the cat LC: a "D-on" cell which fires progressively more rapidly during waking, SWS, and PS, and a "D-off" cell which has the reverse pattern. While Hobson etal., (1976) postulate that 60% of LC

neurons are of the "D-off" type, Chu and Bloom (1974) suggest that only 25% are within this category. Nevertheless, recently cell activity resembling that of the "D-off" type has been found in the rat (Aston-Jones and Bloom, 1980).

The decrease in discharge of LC neurons would indicate that by turning off, the LC is allowing PS to occur. Indeed, Hobson etal., (1975) have shown that just prior to the occurrence of PS, during the period that the "D-off" cells begin to decrease firing, there are cells within the gigantocellular tegmental field (FTG) which increase their discharge rates. As PS nears completion the FTG cells begin to reduce their activity and the LC regains increased firing rates. This mirror image firing rate pattern among two brainstem neuronal groups has led to the formulation of the reciprocalinteraction model for PS generation (McCarley and Hobson, 1975; Mccarley, 1980). The model postulates that the NE containng cells of the LC exert an inhibitory control over the cholinergic FTG. This control is lifted when LC activity becomes low and FTG neurons are allowed to increase discharge rates thereby, producing PS. One possible implication of this model is that changes in activity brought about alteration in sensitivity of LC-FTG cells would trigger PS.

Pharmacological evidence supporting the model is derived from studies in which a decrease in NE, brought about by AMPT, increases PS, while increases in NE, induced by MAO inhibitors, decrease PS (Steriade and Hobson, 1976). Moreover, Vivaldi etal., (1980) have shown that infusions of propranolol, a beta blocker, into the FTG, increases PS. Presumably the propranolol blocks the LC

inhibitory control on the FTG, thus freeing the FTG to generate PS.

Summary

Evidence derived from pharmacological, neurophysiological and lesion studies indicate that (1) the LC may be permitting PS to occur by lifting its control on the FTG cells, (2) it may be involved in PGO activity, and (3) through the magnocellularis nucleus it may be involved in generating the motor inhibition during PS.

Acetylcholine, the FTG cells and PS

The role of acetylcholine in initiating and maintaining arousal has been known for some time (Jouvet, 1975). Moreover, very early on Jouvet (1962) hypothesized that cholinergic mechanisms my be responsible for PS. Converging evidence from pharmacological, electrophysiological, and histochemical studies support Jouvet's prediction and there is speculation that the cholinoceptive FTG cells may play a critical role in initiating the various tonic and phasic aspects of PS.

Increase in acetylcholine availability

Firm evidence that Ach may be involved in arousal and PS is provided by studies which show alterations in Ach levels as a function of EEG activation. For instance, cortical desynchronization induced by stimulation of the mesencephalic reticular formation releases Ach into the cortex (Szerb, 1967; Celesia and Jasper, 1966; Collier and Mitchell, 1967). An increase in striatal Ach is also reported during normal EEG synchronization during normal sleep is accompanied by a decrease in Ach at the cortical level (Celesia and Jasper, 1966; Beani etal., 1968), and within the striatum (Gadea-Ciria etal., 1973). With

respect to PS, increased Ach is found during PS in cortex (Celesia and Jasper, 1966) and striatum (Gadea-Ciria etal., 1973) of normal cats, and in ventricular perfusates of conscious dogs (Haranath and Venkatakrishna-Bhatt, 1973).

Additional evidence linking Ach with cortical activity is provided from studies in which administration of Ach into the carotid artery induces a state of cortical desynchronization (Monnier and Romanowski, 1962). This effect can also be elicited by administration of the acetylcholinesterase inhibitor, physostigmine (Domino etal., 1968).

Aside from their effects in initiating arousal, Ach potentiating agents also increase PS. For example, in chronic pontile or reserpinized cats physostigmine significantly increases PS (Jouvet, 1962, 1975; Karczmar etal., 1970). The PS facilitory effect is also seen in humans (Sitaram etal., 1976; Sitaram etal., 1977), and normal cats (Domino etal., 1968). In fact, in humans physostigmine decreases the latency of both the first and second PS periods without altering their duration (Gillin etal., 1978). This latter finding is particularly interesting since it suggests that cholinergic mechanisms may control the periodicity of PS rather than its duration. Similar effects are also seen with arecholine, a muscarinic receptor agonist (Sitaram etal., 1978).

The possibility that brainstem cholinergic mechanisms may be responsible for these PS effects is suggested by studies where application of cholinergic potentiating agents directly into the pontine reticular formation, or intravenously in pontile cats, elicits both the tonic and phasic components of PS (Mitler and Dement, 1974;

Pompeiano, 1980; George etal., 1964; Baxter, 1969; Amatruda etal., 1975). That the PS inducing areas is localized to tne FTG cells is suggested by extensive findings by Vivaldi etal., (1980). In fact, in studies where Ach agonists are applied directly to the FTG neurons or surrounding areas it is not unusual to see direct transition from waking to PS (narcolepsy).

Decrease in acetylcholine availability

Acetylcholine can be depleted by hemicholinium-3 which blocks choline reuptake, and by atropine which blocks muscarinic receptors. Both drugs selectively suppress PS. For example, atropine abolishes both the tonic and phasic aspects of PS (Jouvet, 1975). This is seen even in PS deprived cats (Henriksen etal., 1972). Hemicholinium-3 administered intraventricularly also abolishes PS (Hazra, 1970; Domino and Stawiski, 1971). Administration of scopolamine, a muscarinic blocker, in humans, delays onset of PS and lengthens the interval between PS episodes (Sitaram etal., 1978; Sagales, 1969, 1975). Further, scopolamine also blocks the PS potentiating effects of arecholine (Sitaram etal., 1978).

To summarize, pharamacological evidence indicates that ach plays a dominant role in initiating arousal and PS.

Acetylcholine, FTG and PGO spikes

While serotonin and NE exert inhibitory control over PGO spikes, it appears that Ach may facilitate PGO. For instance, atropine significantly reduces the frequency of PGO bursts and also blocks PCPA induced PGO (Jacobs etal., 1972). Atropine also significantly reduces reserpine induced PGO (Delorme, 1966). Physostigmine, on the other

hand, is able to restore PGO after its suppression by atropine in a PCPA treated animal (Jacobs etal., 1972; Roch-Monachon, 1976), and it enhances the frequency of PGO in a reserpine treated cat (Delorme, 1966). Moreover, in collicular or pontine transected cats, physosotigmine is able to trigger PGO bursts coupled with rapid eye movements (Magherini etal., 1971); atropine is able to block physostigmines effects.

It is not clear exactly how the FTG may be involved in generating PGO, but data from single-unit studies show increased discharge in FTG just prior to the occurrence of PGO bursts (Steriade and Hobson, 1976; McCarley etal., 1978).

Acetylcholine, FTG and hippocampal theta

Regular 6-12 Hz waves can be obtained from the dorsal hippocampus during waking, PS and surgical anesthesia (Vanderwolf, 1969; Winson, 1972). This waveform, termed theta activity, can be further divided into two categories depending upon the behavior of the animal, and response to atropine (Vanderwolf etal., 1978). The first type of theta (4-7 Hz) occurs during alert total immobility, inter-twitch intervals of PS and anesthesia (urethane, ether). It also occurs when the animal exhibits what has been termed Type II behavior, such as face-washing, shivering, chattering the teeth and tremor. This type of theta is abolished by anti-muscarinic drugs such as atropine, and stimulated by physostigmine. The second type of theta (7-12 Hz) occurs when the animal exhibits Type I behaviors such as walking, running, rearing, shifts of posture and head movement. It also occurs during the PS phasic twitches and it is not abolished by

anti-muscarinic drugs. This type of theta is disrupted by anesthetics (ether, urethane) and morphine.

With respect to the anatomical organization of the systems responsible for generating theta, it may be that theta activity detected in the CA1 and the dentate gyrus formations of the dorsal hippocampus is driven by pacemaker bursting cells located in the medial septal nucleus and the diagonal band of Broca (Petsche etal., 1962, 1965; Stumpf etal., 1962). The pacemaker cells in turn depend upon reticular innervation (Vanderwolf etal., 1978). The evidence supporting such a pathway is provided by extensive stimulation (Vanderwolf, 1978), lesion (Anschel and Lindsley, 1972) and autoradiographic (Rose etal., 1976) studies.

Since there are two types of theta, one atropine-sensitive and the other atropine-resistant, it may be that two ascending brainstem systems are responsible for driving the septal pacemaker cells and generating thaeta. It is suggested that the system responsible for atropine-sensitive theta is cholinergic and, in fact, identical to the ascending cholinergic reticular system of Shute and Lewis (1967)(Vanderwolf, 1978; Vanderwolf and Robinson, 1981). Indeed the first acetylcholinesterase containing pathway that was proved to be cholinergic was that arising from the medial septal and diagonal band nuclei and supplying the hippocampus and dentate gyrus (Lewis and Shute, 1978). The evidence that the FTG area my be driving the pacemaker nurst cells is derived from studies in which stimulation of the FTG in rat (Klemm, 1972; Robinson etal., 1977), and cat (Macador etal., 1974) triggers theta. Recently Vertes (1980) has

confirmed these observation. Further evidence indicating that FTG may drive theta is indicated by single-unit studies which show that FTG cells discharge maximally whenever theta is present (Vertes, 1979). Direct projections from the FTG to the septum have been demonstrated (Robertson etal., 1973; Lynch etal., 1973).

The organization of the atropine-resistant theta system, on the other hand, is puzzling particularly since a major catecholamine input to the hippocampus from the LC is not responsible for generating this type of theta (Kolb and Whishaw, 1977; Monmaur and Delacour, 1978). 5-HT also is not involved because extensive depletions of 5-HT using PCPA fail to alter this type of theta (Vanderwolf, etal., , 1978). Morphine (15mg/kg), however, selectively abolishes atropine-resistant theta. Naloxone is able to reverse morphine's effect (Vanderwolf etal., 1978). Ether and urethane also abolish this type of theta (Vanderwolf etal., 1978). It is not known how these agents alter atropine-resistant theta.

In sum, an ascending cholinergic system originating in the FTG may be responsible for triggering the hippocampal theta present during waking and PS.

Acetylcholine, FTG and muscle atonia

The evidence indicating that the FTG and Ach may initiate the muscle atonia during PS is derived primarily from studies in which cats transected at the pre-collicular level display loss of decerebrate rigidity in response to systemic administration of physostigmine (Magherini etal., 1972). Normally such cats display cataplectic episodes only in the chronic condition and Jouvet (1962)

initially noted that these episodes occurred during PS. The fact that such episodes can be triggered in the acute preparation by administration of physostigmine has proved to be useful in identifying the cellular mechanism responsible for generating the muscle atonia during PS.

Using extracellular microelectrodes, Pompeiano and Hoshino (1976) have noted that prior to onset of cataplectic episodes, FTG units display very low discharge rates. When the animal exhibits loss of decerebrate rigidity, the FTG units increase firing significantly. In fact the increase occurs 5-80 seconds prior to onset of postural atonia. The units then resume low discharge levels once the rigidity reappears. They also note that neurons in the LC exhibit discharge patterns which are opposite to that of the FTG. On tne basis of these findings they suggest that the physostigmine induced loss of decerebrate rigidity may be due to reciprocal alteration in discharge patterns between the LC and the FTG. Similar firing profiles have also been obtained in normal, unanesthetized, restraned cats (Hobson, 1980). Further, in normal cats the atonia can be triggered by application of carbachol directly into the FTG and surrounding area (Mitler and Dement, 1974; Amatruda etal., 1975).

It would appear, therefore, that the suppression of postural muscle tonicity which occurs during PS, in chronic decerebrate preparations, and in acute decerebrate cats treated with physostigmine, may be due to increased FTG activity which then activates the descending bulbospinal inhibitory system of Magoun and Rhines (1946).

The discharge pattern of FTG neurons and the reciprocal-interaction model

Data derived from unaesthetized, head restrained cats indicates that the discharge rate of FTG cells is highest during PS (Hobson etal., 1974), and that the increase occurs as much as several minutes before each PS episode (Hobson etal., 1974). Moreover, the FTG cells display an inverse relationship in firing rates with the LC (Hobson etal., 1975; McCarley and Hobson, 1975). These finding have led to the formulation of the reciprocal-interaction model (Hobson and McCarley, 1977), which posits that (1) the cholinoceptive FTG cells are excitatory to themselves and to the noradrenergic LC neurons, and (2) the LC cells are inhibitory to themselves and to the FTG. According to the model, PS is initiated when the inhibitory LC neurons cease firing and thereby allow the FTG neurons to assume high discharge rates. Since the FTG neurons are excitatory to themselves, FTG discharge rate continues to grow and PS is induced. This stage is terminated when the increased FTG activity excites the LC neurons, which then renew their high discharge and inhibit the FTG.

Recently the raphe neurons have been included in the model and along with the LC they are assumed to exert an inhibitory control on the FTG (McCarley, 1980). Neuronal discharge of raphe and LC during the transition from SWS to PS support the model, since both show a selective decrease in activity (McGinty etal., 1973; Aston-Jones and Bloom, 1980; Chu and Bloom, 1973, 1974).

Considerable controversy, however, surrounds the Hobson and McCarley reciprocal-interaction model priincipally because their

studies on FTG firing repertoires were conduted using restrained cats. The evidence derived from freely moving cats (Siegel and McGinty, 1976; Siegel etal., 1979; McGinty and Siegel, 1977; Siegel, McGinty and Breedlove, 1977), and rats (Vertes, 1977, 1979) has shown that while FTG cells increase their output during PS, the unit discharge rate is comparable to that obtained during waking with movement. As such, it has been suggested that activity of the FTG neurons may reflect motor activity instead of being specific to a particular sleep state (Siegel, 1979a, b; Vertes, 1977). Moreover, Sastre etal., (1981) recently demonstrated that selective destruction of FTG neurons with kainic acid does not alter PS. Electrical lesion of the FTG, on the other hand, completely abolish all signs of PS (Jones, 1979; Sastre etal., 1981). Perhaps kainic acid lesion do not destroy all of the 3000-4000 of the FTG cells, and a few remaining cells are sufficient to induce PS.

Nevertheless, the lesion studies suggest that this area is indeed vitally important for PS generation. Further, the single- unit data suggest that since the FTG neurons are activatd during both waking and PS, these neurons may subserve some specific function common to both states. For example, increased FTG activity could be generating hippocampal theta because theta is present during both waking and PS (Vertes, 1980). Alternatively, the FTG may be involved in some function unique to PS. For instance, increased FTG activity as a consequency of cessation of LC neurons may be triggering the muscle atonia during PS (Pompeiano and Hoshino, 1976; Pompeiano, 1980).

Perhaps the strongest evidence favoring the role of the FTG in PS

comes from pharmacological studies. While this evidence has already been summarized (see section on increase in Ach availability) it will be preented now in more detail and in reference to the FTG area in particular.

George etal., (1964) injected oxotemorine or carbachol directly into the nucleus reticularis pontis oralis and caudalis (FTG area according to the terminology of Berman)(Berman, 1968). The cats displayed all the characteristic signs of PS, viz., rapid eye movements, EEG desynchrony, muscle atonia, nictitating membrane relaxation, and miosis of the pupils. These episodes lasted upto 60 minutes and were usually followed by awakening during which the animals were atonic. Administration of atropine was able to block the development of the drug induced PS.

Baxter (1969) placed carbachol crystals in the mesencephalic grey area - not the FTG, and obtained a behavioral pattern that can only be characterized as intensely emotional in that the animals hissed and growled. When the carbachol was placed in or near the ventricles, however, the EEG as well as the behavioral signs of of PS were obtained. Recently Van Dongen (1980) has confirmed the bahavioral observation of Baxter and found that carbachol injection into the pontis oralis area elicited emotional reaction similar to that obtained by Baxter. Van Dongen also found that carbachol injection into the LC and pontis caudalis produced atonia comparable to that seen during PS. No electrophysiological data were taken in this study, however.

Mitler and Dement (1974) injected carbachol into the LC area and

obtained atonia, increased PGO and rapid eye movemennts. Atropine was able to reverse the carbachol induced behavior.

More detailed studies regarding cholinergic stimulation of the FTG have been done by Hobson and McCarley's group (Hobson, 1980; Mccarley, 1980; Vivaldi etal., 1980). In the earliest study (Amatruda etal., 1975) 3 ug of carbachol (using cerebro- spinal fluid as vehicle) was infused at various location in the pontine brainstem: eight injections were in the FTG, one in the LC, and five in the surrounding pontine area. In the FTG, carbachol elicited PS which was 3.5 times the baseline values. In the surrounding area, the level of PS was only 1.8 times the baseline, while in the LC a 50% decrease was seen. Furthermore, the duration of each PS period was significantly longer in the FTG group then in the surrounding area. Also, FTG injections significantly decreased the latency to PS onset. Increasing the carbachol dosage to 9 ug served to increase the duration and freqeuncy of PS. In this study no attempt was made to block carbachol's PS potentiating effects.

In another set of experiments Vivaldi etal., (1980) have shown that 4 ug of carbachol in saline, infused into the FTG by a cannula system produced all the signs of PS. Unlike the experiment by Amatruda etal., (1975) these observation were made in unrestrained cats. Propranolol, a beta blocker, infused into the FTG was also found to increase PS. This may have been due to propranolol blocking the LC's inhibitory effect on the FTG and thus allowing the FTG to increase discharge and induce PS. In this study, iontophoretic administration of carbachol was also found to increase PS. However,

the drugs were not infused into surrounding pontine area; consequencly it is difficult to assess site specific effects.

While these studies provide valuable data regarding the PS potentiating effects of cholinergic agents placed in the FTG, several important pieces of information are still missing. For instance, (1) all of these studies were done using cats, and therefore generalization to other species limited, (2) there is a paucity of evidence regarding site specificity, (3) all studies administered the cholinergic agents acutely, and (4) the investigators observed the animals during the brief period immediately following the drug administration.

The last two points are particularly important because by conducting such acute experiment the researchers have failed to adequately test the role of the FTG in PS. A long-term chronic drug infusion study, on the other hand, could test such a role. One crucial hypothesis that might be examined in a chronic infusion study involves determining whether prolonged drug- induced changes in excitability of FTG neurons produce concomitant long-term altrations in PS. Additionally, if a PS change is seen it is important to determine whether the change persists throughout the life of the animal or whether PS returns to pre-drug levels once the infusions have stopped and the drug catabolized. Other critical tests that might be undertaken include determining whether chronic drug treatment induces changes in receptor sensitivity which might then influence PS. Indeed, there is evidence that receptor super-sensitivity develops if neurotransmitter availability at post-synaptic receptor or effector

organs is interrupted for a long time (Sharpless, 1964). In fact, Sitaram etal., (1979) have provided data that such changes in receptor-sensitivity may underlie scopolamines PS potentiating effect.

In sum, there is no chronic study which has stringently tested the viability of the hypothesis that the FTG plays a dominant role in PS generation.

Summary

Even though cholinergic mechanisms have been implicated in arousal and PS for quite some time, specific research designed to elucidate these mechanisms has lagged far behind similar research in catecholamines and 5-HT system. However, recently there has been renewed interest in this area and this due primarily to the single-unit studies of Hobson and McCarley which have implicated the FTG cells as generators of PS.

The reciprocal-interaction midel, however, is controversial because it has been found that FTG neuronal activity is not specific to PS but rather may be movement dependent. Nevertheless, considerable interest in this area persists since acute administration of cholinomimetic agents into this region has been shown to induce all the major tonic and phasic components of PS. These acute pharmacological experiment, however, do not adequately test the role of the FTG in initiating PS. Chronic infusion studies, on the other hand, can provide more detailed data regarding long-term changes in PS in response to chronic drug infusion. Such a study is essential because if one posits that PS is due to increased changes in FTG

excitability then long-term changes in PS should be noted in response to chronic availability of cholinomimetic agents in the FTG.

Rationale for a chronic drug infusion study

After years of extensive research the neurochemical basis of PS generation still remains obscure. Even though important gains have been made in identifying the mechanisms underlying the various tonic and phasic aspects of PS, it is still unclear from where within the pontine brainstem the signal triggering the PS phenomenon originates. Much converging evidence seems to favor the FTG cells as the source of this signal, and these neurons have been incorporated in a model which predicts PS onset and rhythmicity.

Despite the considerable controversy surrounding the reciprocalinteraction model for PS generation the model nevertheless has one very important feature: it is a mathematical model and therefore provides a convenient basis upon which detailed analysis of the PS phenomenon can be taken.

The central postulate of the model is that PS can be artificially induced or disrupted by simply altering the excitability of the FTG neurons. While acute pharmacological experiments have provided evidence in support of the model, such experiments have not tested key aspects of the model. For example, one critical hypothesis which has not been tested is whether chronic drug-induced changes in FTG neuronal excitability produce concomitant long-term alterations in PS. Several corollaries arising from this hypothesis which might be tested in a chronic drug infusion experiment include: (1) Are altered PS levels due to changes in PS frequency or duration, or both? (2) Do

the PS alterations persist once the drug has been catabolized? (3) Does chronic drug infusion produce changes in receptor sensitivity which might be detected as a result of alterations in PS? (4) Are these effects site specific?

The purpose of this dissertation is to design a series of experiments, in rats, to test the above hypotheses. A secondary purpose of the dissertation is to assess the effects of such pharmacological manipulations on memory. It is anticipated that perhaps such manipulations may influence memory since there is considerable evidence to indicate that activation of neuronal pathways during PS may serve to facilitate information processing. Evidence linking PS with memory consolidation is presented in the next section.

PS and memory consolidation

A number of researchers have examined the role of sleep in memory, and much converging evidence now exists to suggest that sleep, primarily PS, is actively involved in memory storage processes (for review see Fishbein and Gutwein, 1981). Much of this evidence is derived from studies in which subjects deprived of PS exhibit amnesia of the learned response. Additionally, a PS augmentation is seen following learning (for review see, Fishbein and Gutwein, 1981). This evidence suggests a possible biological role for PS.

In one of the early studies, Fishbein (1970) examined the role of PS in the fixation phase of the consolidation of a long- term memory (LTM) trace. He deprived mice of PS for either 1 or 3 days and then trained them in a one-tiral inhibitory avoidance task. The mice were

then tested for retention of the inhibitory response at various intervals following training. Such a time- dependent testing paradigm allowed for the investigation of shortterm memory (STM) (upto 1 hour) or LTM (1-7 days) deficits. He noted that mice administered 3 days PS deprivation (PSD) demonstrated significant retention deficits when tested at a later time (ie., LTM deficits) but not when tested immediately after training. Moreover, he found that the degree of pre- training PSD was critical because 1 day PSD did not produce deficits in either STM or LTM. These findings suggest that PS is involved in the transfer of information from STM to LTM. Sagales and Domino (1973) and Stern (1971) have replicated these findings.

In another experiment designed to further elucidate the effects of pre-training PSD on the fate of the memory trace, Linden etal., (1975) administered 3 days PSD and then examined the effects of ECS administered at various intervals following training on retention of the inhibitory response 3 days following training. Normally, ECS alone produces a time dependent gradient of retrograde amnesia in that retention increases as the time between training and ECS increases (McGaugh, 1966). However, Linden etal., (1975) noted that the effect of pre-training PSD was to increase the time during which the momory trace was susceptible to ECS disruption. In short, pre-training PSD extended the period of memory lability beyound its normal limits.

Other experiments assessed the effects of post-training post-training PSD on memory maintenance. Fishbein etal., (1971) trained mice in an inhibitory avoidance task, administered 3 days PSD, and then gave ECS at various intevals following termination of PSD. He

noted that on retention testing 1 day following termination of PSD, mice given ECS immediately following PSD displayed amnesia, whereas if the ECS is administered without any PSD no amnesia is seen. This finding clearly demonstrates that PSD serves to hold a newly formed memory trace in a labile form such that ECS administered 3 days after PSD disrupts it. Further confirmation that the labile trace is stabilized during subsequent recovery from PSD is seen in the group where delayed administration of ECS following PSD did not produce amnesia. These finding indicate that PS may serve to reduce the time period during which the memory trace is held in a labile form. These findings have been replicated by Wolfowitz and Holdstock (1971) using rats and an active avoidance task. Additional confirmation is provided by studies in which protein sysnthesis inhibitors produce PSD and block memory storage (Gutwein etal., 1979). Finally, the cholinergic system may be mediating the PSD effects on memory since physostigmine has been reported to reverse the memory disruption induced by PSD (Skinner eta., 1976)

Perhaps the most pervasive finding to emerge from this research is that a PS augmentation is seen following an acquisition of a response. Lucero (1970) was the first to demonstrate that rats trained in a labyrinth like maze displayed a significant increase in PS during the period following training. Subsequently, other researchers have also found similar results. For example, Bloch and his colleagues (Hennevin etal., 1971; Leconte and Hennevin, 1973; Leconte etal., 1973), and Smith etal., (1974) nave also noted the PS augmentation following learning, but more importantly, they

have shown that once the animals reach the asymptote of the learning curve, the PS augmentation ceases and PS returns to baseline levels. These findings have been extended by Kitahama (1973) who has shown that the PS augmentation is related to the complexity of the learning task since animals trained in a single compartment Y-maze do not exhibit as great an augmentation in comparision to animals trained in a more difficult maze. Further confirmation is provided by Fishbein etal., (1974) who have shown that mice trained in an active avoidance task display PS increases during the 24 hours following learning. In this study the most interesting finding was that 'slow' learners demonstrated the greatest PS augmentation. Perhaps the 'slow' learners had difficulty integrating the new and complex information and as such a greater demand was placed on the animal's information processing system. This in turn may have activated a neural chain of events designed to facilitate the acquistition of the response and the PS augmentation is an indicator of the general activation.

In summary, both PSD and PS augmentation studies provide firm evidence to indicate that the nature of the neuronal events triggered during PS provide an atmosphere conducive to memory processing. Considering that PS is triggered from within the pontine brainstem, it is tempting to speculate that alterations in PS induced by changes in activity of the PS generator may in turn influence memory processing. Indeed evidence does exist to support such a prediction. For instance, post-training reticular stimulation facilitates retention if the stimulation is applied within 90 seconds following learning (for review see Bloch, 1977). Reticular stimulation also facilitates

recall of a forgotten maze habit (Sara etal., 1980), and Bloch etal., (1975) have shown that rats trained in a maze fail to show PS augmentation if reticular stimulation is applied following learning. Perhaps the reticular stimulation fullfils the work of PS by triggering the neural chain of events leading to memory consolidation.

While data exist to indicate that electrical stimulation of the reticular formation facilitate memory, no evidence is provided to suggest that long-term pharmacological manipulation of the pontine PS generator produces changes in memory. Further, it is important to ascertain whether such pharmacological manipulation produce concomitant changes in PS in that if PS is disrupted are there similar alterations in memory? Considering that the cholinergic system has been postulated to play a major role in PS it is important to determine if Ach induced activation of neuronal pathways produce changes in memory. Therefore, the second purpose of this dissertation is to design a series of experiments to test whether cholinergic stimulation of pontine nuclei produce changes in memory concomitant with changes in PS. The following section provides evidence linking the cholinergic system to memory.

Acetylcholine and memory

In comparision to the other neurotransmitter systems, the cholinergic system is perhaps the most strongly implicated in memory, and the evidence suggests that in general Ach agonists facilitate memory while Ach antagonists disrupt it (Hunter etal., 1978). Quite possibly the most important evidence to emerge from research in this

area is that (1) the nature of the memory deficits induced by Ach antagonists is remarkably similar to the memory disorders seen in normal aged and Alzheimer's patients (Drachman and Leavitt, 1974; Wurtman, 1979), and (2) in normal adults and aged animals, treatments with Ach agonists improves memory (Bartus etal., 1980; Davis etal., 1978; Sitaram etal., 1978). In view of these findings the potential usefulness of cholinergic drugs as a therapeutic tool is clear; that drugs potentiating cholinergic transmission might ameliorate the memory disorders associated with aging and senile dementia (Davis and Yamamura, 1978; Bartus etal., 1982).

Increasing availability of Ach

Much of the evidence linking Ach to memory is derived from the systematic series of experiments conducted by Deutsch and his group (1971). For example, in one experiment (Wiener and Deutsch, 1968) it has been shown that bilateral intra- hippocampal injections of DFP, an irreversible anticholinesterase, 28 days following training facilitates recall of a learned maze escape habit. Similar injections at 7- 21 days, however, produce memory deficits. The results have been confirmed by others (Squire, 1970). Deutsch contends that these data support his theory that the cholinergic synapse is the site of memory, and that excitability in these synapses increases following training upto a certain level and then declines; poor memory is, therefore, associated with a loss in synaptic excitability.

Such a theory has profound implications because it postulates that memory loss can be alleviated by renewing availability of Ach which then increases synaptic excitability and reactivates the memory trace.

Indeed memory and histochemical data from aged animals and humans support such a contention. For instance, there is histochemical evidence that a 40-60% decline in the Ach synthesizing enzyme choline acetyl- transferase (CAT) occurs in the cortex as a function of increasing age in humans (McGeer and McGeer, 1975). Similar age-related CAT losses have been reported in the hippocampus of humans (Perry etal., 1977) and mice (Vijayan, 1977). AchE decreases in cortical regions of humans has also been noted (McGeer and McGeer, 1975). In the rat it has been reported that AchE levels are highest in mature animals and then a decrease is seen as a function of age (Moudgil and Kanungo, 1973). Vijayan (1977) also reports a significant decline in AchE in the hippocampus of aged mice. Choline uptake, another indicator of central cholinergic activity, also undergoes a systematic age- related decrease (Sherman etal., 1980).

With respect to memory, Bartus (1979) has examined the effects of physostigmine on memory of young (3-7 years) and aged (18+ years) rhesus monkeys. He notes that young monkeys show memory facilitation at moderate doses while higher doses disrupt memory. Old monkeys, on the other hand, display memory facilitation at the higher doses. Perhaps the higher doses of physostigmine are needed to adequately replenish Ach availability in old monkeys. In another study Bartus etal., (1980) administered choline-rich and choline-deficient diets to young and aged mice for 4.5 months and then assessed the effects of such manipulations on memory. They note that old mice (23 months and older) display significant deficits in retention of the learned task. Yet old mice placed on choline-enriched diets perform as well as young

control mice, while young mice placed on choline-deficient diets perform like senescent mice.

In aged humans, the effects of increasing Ach are, however, less clear. For example, Thal etal., (1981), in a double blind crossover study, administered choline chloride to seven mild to moderately demented patients. No memory improvements were seen despite a considerable increase in plasma choline levels. Similar results were obtained by Mohs etal., (1980).

In normal adults, intravenous infusions of physostigmine significantly enhance both storage and retrieval of LTM; STM is not affected however (Davis etal., 1978). Administration of arecholine, choline and lecithin (phosphotidylcholine, an Ach precursor) has also been reported to facilitate human serial learning (Sitaram etal., 1978; Gillin etal., 1981). In fact in these studies 'poor' performers showed significantly more facilitation then 'good' performers. Perhaps the 'poor' performers displayed low cholinergic synaptic excitability and potentiation of Ach helped facilitate transmission of information. Indeed it has been reported that there is increased CAT in frontal and temporal cortex of one strain of mice with high maze learning ability in comparision to mice that were poor learners (Mandel and Ebel, 1974).

With respect to studies in animals, physostigmine facilitates the retention of an appetitive maze-learning habit (Stratton and Petronovich, 1963). Nicotine at low doses has been found to facilitate pole- jump avoidance response; higher doses, however, disrupt the behavior (Domino, 1965). Other researchers have also found that both

pre-and post-trial administration of nicotine facilitates performance of a variety of tasks (Bovet etal., 1966; Bovet-Nitti, 1969; Garg and Holland, 1968, 1969; Garg, 1969; Batig, 1970; Evangelista etal., 1970; Erickson. 1971).

Decreased availability of Ach

Scopolamine disrupts the acquisition of a variety of active avoidance, passive avoidance, and maze learning tasks (Herz, 1960; Whitehouse, 1964; Pazzagli and Pepeu, 1964; Meyers, 1965; Dilts and Berry, 1967; Alpern and Jackson, 1978). Scopolamine has also been found to disrupt retention of pole-jump avoidance response (Gruber etal., 1967). and passive avoidance response in both rats and mice (Bohdanecky and Jarvik, 1967; Calhoun and Smith, 1968). Moreover, Glick and Zimmerberg (1971) have noted that scopolamine induces retrograde amnesia when administered upto 1 hour following training.

In normal adults, scopolamine impairs human serial learning (Sitaram etal., 1978); a subsequent administration of arecholine is able to reverse the effect of scopolamine. Scopolamine, in fact, has a more potent disruptive effect on 'poor' learners than on 'good' learners. It would appear that scopolamine fails to block memory in 'good' learners because in this group there may be cholinergic synaptic excitability sufficient enough to process information. In 'poor' learners, on the other hand, scopolamine may depress further an already diminished synaptic excitability such that no information is processed. It must be pointed out that both 'poor' and 'good' learners were significantly worse then placebo; it is just that in 'poor' learners tne effects of scopolamine are exacerbated.

Additional evidence to suggest that a decline in cholinergic activity impairs memory is provided by Drachman and Levitt (1974). They have demonstrated that the memory deficit induced by scopolamine in young subjects resembles the deficits seen in normal aged subjects. Recently, Drachman (1977) has shown that scopolamine induced memory impairments in young adults can be reversed by subsequent administration of physostigmine. This is confirmed by Sitaram etal., (1978) who have shown that arecholine also reverses the effects of scopolamine in normal adults. These data coupled with the finding that Ach potentiation in aged animals restores memory suggests that cholinergic potentiating drugs may reverse the memory deficits seen in normal aged patients (Davis and Yamamura, 1978). Such treatment may also benifit patients with Alzheimers disease and other senile dementias since these patients also display severe memory impariment and a decrease in CAT and AchE in neocortex, hippocampus and striatum (Davies and Mahoney, 1976; Perry etal., 1977; Reisine etal., 1978; Spillane etal., 1977; Whitehouse etal., 1982).

With respect to the nature of the anatomical pathway it is reasonable to assume that the cholinergic influences on memory may be mediated by the ascending cholinergic system of Shute and Lewis (1967) innervating the limbic system, in particular the septal-hippocampal structures, and the cerebral cortex. Evidence supporting such a function for this pathway is provided by studies in which (1) reticular stimulation facilitates memory (Sara etal., 1981; Bloch 1977) and releases Ach in cerebral cortex (Szerb, 1967), and (2) lesion and stimulation of hippocampus and other limbic structures

profoundly affects memory consolidation (for review see Izquierdo, 1975). Perhaps activity in the ascending cholinergic system determines the rate of firing of hippocampal cells (Ranck, 1975) and association cortical interneurons (Steriade, 1978), which in turn may influence the durability of the memory trace. Additionally, the activation of this pathway during PS may also serve to influence the neuronal events responsible for memory consolidation (Fishbein and Gutwein, 1981). To test this latter possibility it is important to determine if chronic cholinergic stimulation of pontine areas trigger PS and also influence memory. Therefore, the second aspect of this dessertation assesses the effects of long-term cholinergic stimulation of the FTG on memory. Considering the evidence, it is anticipated that cholinergic agonists (eg., carbachol) will enhance activity in this pathway thereby leading to increased PS and memory facilitation. Cholinergic blockade, on the other hand, induced by scopolamine should depress PS and block memory.

GENERAL METHOD

Subjects

Male Holtzman rats weighing 400 gms are housed singly in wire-mesh rat cages with food and water available ad libitum. A 12 hour light-dark cycle (7 AM- 7PM light on), and constant temperature (74°F ± 2) are maintained throughout the experiment.

Electrode and cannula implant procedure

The animals are implanted with chronic indwelling EEG and EMG electrodes under Nembutal anesthesia. The EEG electrodes consist of four stainless steel screws secured to single stranded stainless steel wire. The screws are positioned one on either side of the mid-sagittal sinus, two each anterior to lambda and bregma. This allows for EEG activity to be recorded contralaterally between the anterior and posterior cortical electrode pairs. Two EMG electrodes, made of multi-stranded stainless steel wire soldered to male Amphenol connectors, are sewn into the nuchal muscles. The six Amphenol connectors are positioned atop the animal's head and secured to the skull wth dental cement.

At this time an L-shaped cannula assembly is prepared. The cannula assembly consists of an L-shaped (1.5 cm length) 21 gauge stainless steel cannula attached to a 1.5 cm piece of vinyl tubing. The cannula assembly is filled with .9% saline and after it is examined to determine that no air bubbles are trapped in it, the vinyl tubing is heat sealed. The cannula is lowered into the brain and secured with dental cement. The end result is that the vinyl tubing

connecting the cannula is sitting on the surface of the skull.

Alzet mini-pump implant procedure

An Alzet osmotic mini-pump (Alza Corp) is used to infuse the pharmacological agents into discrete regions of the brainstem. The salient feature of this method of drug delivery is that small quantities of drug can be infused at a constant rate (0.5 ul/hr or 1.0 ul/hr) over a period of seven (model 1601, 1701) or 14 (model 2002) days.

At the time of pump implant the animals are lightly anesthetized with ether, a small incision made in the skin, and the underlying vinyl tubing protruding from the L-shaped cannula exposed. The vinyl tubing is cut to about 1 cm, and the pump connected to the tubing. Extreme care is exercised during this phase of the implant to insure that no air bubbles are trapped in the tubing. The pump is secured to the nuchal muscles with two sutures to prevent it from becoming dislodged. The skin is then sutured, thus placing the pump and vinyl tubing under the skin. Topical antibiotic is applied to the skin to prevent infection.

Histology

Histological verification of cannula placement consists of examining 40 um thick frozen sections cut using the frozen method. The sections are stained using cresyl violet, and cannula placement verified using the rat brainstem atlas of Palkovits and Jacobowitz (1974).

EXPERIMENT 1

Introduction

There is evidence to indicate that acute administration of Ach potentiating agents into the pontine brainstem produce all the tonic and phasic components of PS. The purpose of this experiment is to determine whether long-term cholinergic manipulation of pontine structures produces sustained alterations in PS.

Subjects

Fifty-one male Holtzman rats weighing 400 gms are used. One week prior to surgery the animals are moved to clear plexiglas cages (12 in x 12 in x 20 in) with wood shavings serving as bedding. Throughout the experiment the animals are housed singly in these cages, and food and water are available freely. In addition, a 12 hr light dark cycle and constant temperature are maintained.

Experimental Design

The animals are randomly assigned to one of three cannula placement groups: (A) at the level of the genu of seventh cranial nerve (B) caudal to the seventh cranial nerve and (C) fourth ventricle.

Within each group the animals are randomly assigned to one of three drug conditions: (i) carbachol (0.5 ug/1 ul saline/hr), (ii) scopolamine (9.0 ug/1 ul saline/hr), and (iii) saline (0.9%/hr). carbachol and scopolamine are dissolved in 0.9% normal saline.

Procedure

One week after electrode and cannula implantation the animals are

moved to a sound attenuated, electrically shielded, walk-in recording chamber for the purpose of obtaining polygraphic recordings of the sleep-wake cycle.

The animals are allowed two days to become adapted and acclimated to the recording chamber and recording cables. The recording cables consist of a ball-bearing commutator mounted on a counter-balanced arm permitting the animals unrestricted activity.

Following the adaptation period, at 9 AM, the polygraph (Grass Model 79B) is switched on and a 24 hr baseline recording obtained. At 9 AM the following morning the animals are disconnected from the recording cables and the Alzet mini-pump implanted. Immediately after pump implant the animals are reattached to the recording cables and 9 consecutive days (7 days drug, 2 days post-drug) of polygraph data are obtained.

Throughout the recording session the animals are not disturbed except to replenish food and water. However, the behavior of the animals is monitored via a closedcircuit television system.

After 9 days of recording the animals are disconnected from the polygraph and removed from the recording chamber for two weeks. During the two weeks the wood-shavings are changed every three days and food and water is freely available. Subsequent to this the animals are returned to the recording chamber and allowed 2 days to adapt to the recording cables. At 9 AM the next morning a 24 hr post-experimental baseline recording is obtained.

Subsequently the animals are administered a lethal dose of Nembutal and decapitated. The brains are removed and placed in 10%

Formalin for later histological examination of cannula placement.

Data analysis

The polygraphic sleep-wake records are scored manually for awake, SWS, and PS. Each stage is identified on the basis of strict electrophysiological and behavioral criteria. Wakefulness is characterized by the presence of desynchronized EEG and an active EMG. SWS is noted when the EEG displays high voltage slow waves and the EMG exhibits a decreased level of activity relative to wake. PS is noted when the EEG shows cortical desynchrony coupled with EMG suppression, while behaviorally the animal must display muscular flaccidity; whisker, ear and body twitches; and irregular respiration.

The data are analyzed for: number of episodes of awake, SWS, and PS; time spent in awake, SWS and PS; total sleep time (TST) (SWS+PS); percent PS and percent SWS. Repeated measures analysis of variance is used to test within group differences across drug infusion days. Additional analyses consisting of matched and independent T-tests, and non-parametric statistics are also used.

Results: Experiment 1

On the basis of histological examination the animals are divided into three general groups: (A) at the level of the genu of the seventh and sixth cranial nerves, (B) caudal to the genu of the seventh nerve, and (C) fourth ventricle. The cannula placement for groups A and B is schematized in Fig 1-2, and for group C the cannula placement is in the fourth ventricle at the level of the genu of the seventh nerve.

It is noted that the following days are pooled within subjects: (i) days 2 through 5, and (ii) days 6, 7, post-drug 1 and post-drug 2. This is done to reduce the variance. Moreover, the first 24 hours immediately following pump implant are analyzed separately. Whenever there are no differences between baseline and post-experimental baseline, these values are pooled and all comparisions are made to these pooled baseline values. It must be noted that all pooling is within-subjects, and in only one instance is there a difference between baseline and post-experimental baseline. In such a situation comparision is to baseline values.

GROUP A: AT THE LEVEL OF THE GENU OF THE SEVENTH NERVE

This group is divided into three drug categories: (1) Carbachol (n=5),(2) scopolamine (n=5), and (3) saline (n=5). The cannula placement for each category is similar and is schematized in Fig. 1.

24 hr cycle (excluding the first 24 hrs following pump implant)

Initially the data are analyzed for overall differences in sleep patterns during 24 hr blocks. Table 1 summarizes the percent total sleep time (TST) for each drug category during the drug infusion days.

A 2-way repeated measures ANOVA with test for simple effects reveals no significant differences between drug categories during baseline and post-experimental baseline; there are no within group differences either. Therefore, these values are pooled. Scheffe post-hoc comparisions reveal no significant differences between pooled baseline and days 2 through 5, and the next four days.

Table 2 summarizes the percent slow wave sleep (SWS) of total time. There are no significant differences between groups during baseline and post- experimental baseline. The pooled baseline scores are not significantly different.

Figure 3 summarizes the percent PS deviation from pooled baseline during each drug treatment. Within the carbachol drug category there is a significant 36% increase in PS over pooled baseline values during days 2 through 5 of drug infusion ($F=10.88$; $df=3,36$; $p<.05$). During the subsequent four days, however, the PS levels return to baseline.

Scopolamine, on the other hand, produces a significant 52% decrease in PS during days 2 through 5 ($F=42.68$; $df=3,36$; $p<.01$). During the subsequent four days there is still a significant 41% PS decrease ($F=26.92$; $df=3,36$; $p<.01$). No significant alteration in PS is found with saline.

Figures 4, 5 and 6 summarize the 24 hr PS rhythmicity cycles as a function of carbachol, scopolamine and saline infusion.

In order to discern the portion of the 24 hr sleep rhythm when the PS alterations occur, the 24 hr blocks are divided into DAY-NIGHT cycles (7 AM to 7 PM, day; 7 PM to 7 AM, night). Again 2-way repeated measures ANOVAS with tests of simple effects and Scheffe post-hoc

comparisions are used.

Table 3 summarizes the percent TST for the day and night cycles, separately. There are no significant differences between drug conditions in baseline or post-experimental days. Pooled baseline scores also reveal no significant changes in TST during treatment conditions.

Table 4 summarizes the percent SWS for the day and night cycles, separately. There are no significant differences between carbachol, scopolamine, or saline during baseline or post-experimental baseline. Further, comparisions between pooled baseline scores and days 2 through 5, and days 6 through post-drug 2 reveal no alteration in SWS.

Day Cycle

Figure 7 summarizes the percent PS deviation from pooled baseline values during the day cycle. There is a non-significant 15% increase in PS with carbachol during days 2 through 5. During the subsequent four days there is a non-significant 13% increase. Saline does not significantly alter PS levels during the day cycle. Scopolamine, on the other hand, produces a significant 61% decrease in PS during days 2 through 5 ($F=31.89$; $df=3,36$; $p<.01$), and a 40% PS decrease during the remaining 4 days ($F=13.34$; $df=3,36$; $p<.01$). Figure 8 summarizes alterations in PS induced by scopolamine during each day cycle recording session in the experiment (Day 1 is excluded). Dunnett's test for comparisions involving a control mean (baseline) reveal that, except for post- experimental baseline, all days are significantly different from baseline. In fact, even during post-drug 1 and 2, a

period when the pump should have ceased operating, PS levels are 35% below baseline.

In order to assess the nature of the PS alterations, the data are separately analyzed in terms of changes in frequency and duration of wake, SWS and PS episodes.

Table 5 summarizes the mean frequency of awake episodes. Separate Chi- square tests for carbachol, scopolamine, and saline reveal no changes in the number of awake episodes as a function of drug or saline infusion.

Table 6 summarizes the mean frequency of SWS episodes. There are no significant alterations in the frequency of SWS episodes.

Table 7 summarizes the frequency of PS episodes. Once again there is no significant change in the frequency of PS episodes. It must be noted that in the scopolamine condition, there is a 41% decline in PS episodes during days 2 through 5. This is not significant, however.

Table 8 summarizes the mean duration of wake episodes. A 2-way repeated measures ANOVA with tests for simple effects and post-hoc Scheffe comparisions reveal no significant changes.

Table 9 summarizes the mean duration of SWS episodes. In the carbachol and saline conditions there are no significant alteration in SWS duration. Scopolamine, however, increases the length of SWS episodes by 37% during days 2 through 5 ($F=16.58$; $df=3,36$; $p<.01$), and by 32% during the next four days ($F=12.06$; $df=3,36$; $p<.01$).

Table 10 summarizes the mean duration of PS episodes. Saline does not alter mean PS duration. Carbachol, decreases mean PS length by 16% during days 2 through 5 ($F=9.34$; $df=3,36$; $p<.05$), and on days 6

through post-drug 2 there is a 15% decrease which is just short of significance at the 5% alpha level ($F=8.10;df=3,36$;critical F needed to reject, $F=8.28$). Scopolamine reduces mean PS duration by 31% during days 2 through 5 ($F=37.12$; $df=3,36;p<.01$), and on the subsequent four days mean PS length is reduced by 21% ($F=17.65;df=3,36;p<.01$).

Night Cycle

Figure 9 summarizes the percent PS deviation from pooled baseline. Carbachol produces a significant 88% increase in PS during nights 2 through 5 of drug infusion ($F=9.23;df=3,36;p<.05$). During the next four nights PS levels return to normal. Scopolamine, on the other hand, produces a 38% decrease in PS during nights 2 through 5. This decrease, however, is short of statistical at the 5% alpha level ($F=6.48;df=3,36;p<.1$). During the subsequent four nights, however, scopolamine produces a significant 44% decrease in PS ($F=8.76$; $df=3,36;p<.05$). Saline does not significantly alter PS levels.

Figure 10 summarizes the alterations in PS during the night cycle as a function of carbachol infusion. Dunnett's test for comparision involving a control mean reveals that only nights 2 and 3 are significantly different from baseline. However, during nights 4 and 5 there is still a PS augmentation over baseline.

Table 5 summarizes the frequency of wake episodes. There is no change in wake frequency as a result of drug or saline administration. Table 6 summarizes the frequency of SWS episodes. Carbachol or saline do not significantly alter the number of SWS episodes. Scopolamine, however, reduces the SWS frequency by 28% during nights 2

through 5, and by 32% during the next four nights (Chi-square=7.91;df=3;p<.05).

Table 7 summarizes the mean number of PS episodes. Scopolamine and saline do not alter PS frequency by 129% during nights 2-5 (Chi-square=11.16; df=3; p<.02); on the subsequent four nights PS frequency assumes baseline levels.

Table 8 summarizes the mean wake duration. There are no significant alterations. Table 9 summarizes the mean length of SWS episodes. Carbachol and saline do not alter SWS length. Scopolamine, however, increases SWS length, but only during nights 2 through 5 (F=15.17;df=3,36;p<.01).

Table 10 summarizes mean PS duration. Carbachol or saline do not alter mean PS length. However, it must be noted that carbachol tends to shorten PS length during the period of drug administration. Scopolamine significantly reduces PS length during nights 2 through 5 (F=17.21; df=3,36;p<.01).

The first 24 hours of pump operation

Tables 11, 12 and 13 summarize the percent TST, SWS and PS during the day and night cycle. All groups undergo significant reductions in TST, SWS and PS during the day cycle. During the night cycle carbachol significantly increases TST, SWS and PS. Scopolamine does not alter TST, but increases SWS and decreases PS by 44% (F=7.1;df=3,36;p<.05). Saline does not alter the sleep stages during the night cycle. It must be noted though that saline increases PS by 45% but it is not significant (F=3.56;df=1,12;p<.1).

Behaviorally the animals appear mobile and alert about one

half-hour after pump implant, and throughout the experiment no behavioral difference could be discerned before and after pump implant, and between drug and saline conditions.

GROUP B: CAUDAL TO THE GENU OF THE SEVENTH NERVE

This group is divided into the following drug categories: (1) carbachol (n=13), (2) scopolamine (n=5), and (3) saline (n=6). The carbachol group has two sub-divisions depending upon the placement of the cannula: (a) midline ponto-medullary (n=8), and (b) left-side ponto-medullary (n=5). The cannula placement for all groups is schematized in Fig.2.

24 hour cycle (excluding the first 24 hrs following pump implant)

Table 14 summarizes the percent TST. There are no significant differences between groups in baseline or post-experimental baseline. Moreover, comparisions between pooled baseline and days 2 through 5, and days 6 through post-drug 2 reveal no significant differences.

Table 15 summarizes the percent SWS. No significant alterations in this sleep stage could be discerned either.

Figure 11 summarizes the percent PS deviation from pooled baseline. There are no significant differences between groups in baseline or post-experimental baseline. Moreover, comparisions with pooled baseline reveal no significant alterations in PS in either carbachol or saline groups. Scopolamine produces a significant 49% decrease during days 2 through 5 ($F=74.03;df=3,60;p<.01$), and a 38% decrease during the next four days ($F=43.65;df=3,60;p<.01$).

Day Cycle

The data are divided into DAY-NIGHT blocks in order to assess changes in the sleep rhythm. Table 16 summarizes the percent TST during the day cycle. Post-hoc comparisions reveal no significant changes in TST as a function of drug of saline infusion.

Table 17 summarizes the percent SWS of total time. Once again there are no significant alterations in this phase of sleep as a function of drug or saline infusion.

Figure 12 summarizes the percent PS deviation from pooled baseline. While carbachol and saline do not significantly alter PS levels, scopolamine reduces PS by 67% during days 2 through 5 ($F=42.16;df=3,60;p<.01$), and by 47% during the subsequent four days ($F=20.93;df=3,60;p<.01$). Figure 13 summarizes the alterations in PS as a function of scopolamine infusion during each day cycle of the experiment. Dunnett's test for comparision involving a control mean reveals that all days except post-drug 2 and post-experimental baseline are significantly different from baseline.

Table 18 summarizes the frequency of wake episodes. There are no alterations. Table 19 lists the number of SWS episodes. There are no alterations as a result of drug or saline infusion. Table 20 summarizes the frequency of PS episodes. Carbachol or saline do not significantly alter PS frequency. Scopolamine, however, significantly decreases PS frequency by 56% during days 2 through 5, and during the next four days PS frequency is reduced by 40% (Chi-square=11.75; df=3, $p<.01$).

Table 21 summarizes the mean duration of wake episodes. While saline and carbachol do not significantly alter wake duration,

scopolamine significantly increases the length of wake episodes during days 6 through post-drug 2 ($F=9.88;df=3,60;p<.05$). Table 22 lists the mean duration of SWS episodes. Saline does not alter SWS duration. In the left-side ponto-medullary group carbachol does not alter SWS duration. However, in the mid-line ponto- medullary group carbachol decreases the length of SWS episodes during days 2 through 5 ($F=9.11;df=3,60;p<.05$). Scopolamine, on the other had, increases the length of SWS episodes during the period of drug infusion but this is short of significance at the 5% alpha level ($F=7.95;df=3,60;p<.1$).

Table 23 summarizes the mean duration of PS episodes. Saline has no effect. In midline ponto-medullary group carbachol has no effect. However, there is a marked 20% decrease in the left-side ponto-medullary group ($F=13.91;df=3,60;p<.01$). Scopolamine also decreases the length of PS episodes during the period of drug infusion ($F=36.65;df=3,60;p<.01$).

Night Cycle

Table 16 summarizes the percent TST. There are no significant changes. It must be noted however, that scopolamine produces a 30% increase in TST on nights 2 through 5. However, this is not significant ($F=7.41;df=3,60;p<.1$).

Table 17 summarizes the percent SWS. There are no significant changes. Scopolamine produces a 31% increase in SWS during nights 2 through 5, but this is not significant ($F=6.94;df=3,60;p<.1$).

Figure 14 summarizes the percent PS deviation from pooled baseline. In the midline ponto-medullary carbachol group there is 48% increase in PS during nights 2 through 5. However, this increase is

not statistically significant even at the 10% alpha level ($F=5.85;df=3,60;p>10\%$). The 42% increase in PS seen with saline is also not significant. Moreover, there are no significant differences in the other two groups.

Table 18 lists the number of wake episodes. There are no changes. Table 19 summarizes the frequency of SWS episodes. While carbachol and saline do not alter SWS frequency, scopolamine reduces the number of SWS episodes during nights 6 through post-drug 2 (Chi-square7.84;df=3$p<.05$). Table 20 lists the frequency of PS episodes. There is no effect with drug or saline. However, it must be noted that in the midline ponto-medullary group there is a 79% increase in PS frequency over baseline levels. This is not significant though (Chi- square=5.43;df=3).

Table 21 lists the mean duration of wake episodes. Carbachol and saline have no effect. Scopolamine increases the duration of wake episodes by 88% during nights 6 through post-drug 2 ($F=14.2;df=3,60;p<.01$). Table 22 summarizes the mean length of SWS episodes. Carbachol ad saline have no effect. Scopolamine lengthens SWS episodes by 50% during the period of drug infusion ($F=18.26;df=3,60;p<.01$). Table 23 lists the mean duration of PS. There are no alterations.

The first 24 hours of pump operation

Tables 11, 12 and 13 summarize the percent TST, SWS and PS during the day and night cycles. All groups undergo significant reductions in TST, SWS, and PS during the day cycle (except left-side ponto-medullary carbachol which displays a non-significant 7%

reduction in SWS). During the night cycle despite the extensive PS reductions during the day cycle, only the left-side ponto-medullary carbachol shows a significant PS increase ($F=10.7;df=1,12;p<.05$). In fact the midline ponto-medullary group shows a 48% PS decline which is not significant.

GROUP C: FOURTH VENTRICLE

This group is divided into three drug categories: (1) Carbachol (n=4), (2) Scopolamine (n=4), and (3) Saline (n=4). The cannula placement for each category is similar and it is in the fourth ventricle at the level of the genu of the seventh nerve.

24 hr cycle (excluding the first 24 hours following pump implant)

Table 24 summarizes the percent TST. There are no significant differences between pooled baseline and the other days as a function of drug or saline infusion.

Table 25 summarizes the percent SWS. In the carbachol and scopolamine categories there are no significant differences between pooled baseline and drug infusion days. In the saline condition, there is a small 15% increase during days 6 through post-drug 2. This increase is not significant, however ($F=8.14,df=3,27;p<.1$).

Figure 15 summarizes the percent PS deviation from baseline. In the carbachol treatment there is no significant change in PS levels from pooled baseline as a function of drug infusion. Scopolamine, on the other hand, produces a significant 31% decrease in PS during days 2 through 5 ($F=10.06;df=3,27;p<.05$), and a nonsignificant 28% decrease

during the next four days ($F=8.28;df=3,27;p<.1$). In the saline treatment, there is an unexpected significant difference between baseline and post-experimental baseline ($F=10.48;df=3,27;p<.05$). In fact, post-experimental PS levels are significantly higher than even days 2 through 5 ($F=9.05;df=3,27;p<.05$), and days 6 through post-drug 2 ($F=13.32;df=3,27;p<.05$). Therefore, saline comparisions are to baseline levels instead of pooled baseline levels. There is no significant alteration in PS during days 2 through and days 6 through post-drug 2.

Day Cycle

Table 26 summarizes the percent TST. Since there are no significant differences between baseline and post-experimental baseline, these values are pooled. Carbachol and scopolamine do not alter TST significantly. Saline does not produce alterations during days 2 through 5. However, on days 6 through post-drug 2 there is a non-significant 16% elevation in TST ($F=7.49$; $df=3,27;p<.1$).

Table 27 summarizes the percent SWS. Comparisions are with pooled baseline values. In the carbachol and scopolamine condition there is no significant alteration in SWS. Saline, on the other hand, produces a significant 18% increase during days 6 through post-drug 2 ($F=11.01;df=3,27;p<.05$).

Figure 16 summarizes the percent PS deviation from pooled baseline values. Carbachol and saline do not significantly alter PS levels. Scopolamine, however, decreases PS by 40% during days 2 through 5 ($F=11.83;df=3,27;p<.05$), and by 37% during days 6 through post-drug 2 ($F=10.24;df=3,27;p<.05$). Figure 17 summarizes the percent PS

deviation from baseline during each day cycle of the experiment. Dunnett's test for comparisions involving a control mean reveals that except for post-experimental baseline all other PS levels are significantly lower than baseline.

Tables 28, 29, and 30 summarize the frequency of wake, SWS and PS, respectively. There are no effects. Table 31 lists the mean duration of wake. There are no allterations as a result of drug or saline infusion. Table 32 summarizes the mean length of SWS. Carbachol and saline have no effect. Scopolamine, on the other hand, increases the length of SWS episodes by 31% during the period of drug infusion ($F=11.74;df=3,27;p<.05$). Table 33 lists the mean duration of PS. There are no effects.

Night Cycle

Table 26 summarizes the percent TST. In the carbachol and scopolamine conditions, baseline and post-experimental baseline are not significantly different from each other. Therefore, comparisions are to the pooled baseline. Carbachol does not significantly alter TST during nights 2 through 5. However, on night 6 through post-drug 2 there is a non-significant 27% decrease in TST ($F=8.34;df=3,27;p<,1$). Scopolamine does not significantly alter TST either during nights 2 through 5 or nights 6 through post-drug 2. In the saline condition there is a significance difference between baseline and post-experimental baseline. Therefore, comparisions are performed with baseline values. There is no significant difference on nights 2 through 5, and on nights 6 through post-drug 2, even thogh there is a 30% increase in TST ($F=6.19;df=3,27;p>.1$).

Table 27 summarizes the percent SWS. In the carbachol treatment there is no significant difference between pooled baseline and nights 2 through 5. On night 6 through post-drug 2 there is a non-significant 26% decrease in SWS ($F=7.96;df=3,27;p<.1$); scopolamine produces no significant alterations. In the saline condition there is a significant difference between baseline and post- experimental baseline ($F=10.78;df=3,27;p<.05$); therefore, comparisions are o baseline values. During nights 2 through 5 there is no significant alteration in SWS. On the next four nights there is a non-significant 33% increase in SWS ($F=8.32;df=3,27;p<.1$).

Figure 18 summarizes the percent PS change from pooled baseline. Carbachol or scopolamine do not significantly alter PS. In the saine condition there is a significant difference between baseline and post-experimental baseline ($F=19.55;df=3,27;p<.01$). In fact, post-experimental baseline PS is significantly higher than even nights 2 through 5 ($F=16.85;df=3,27;p<.01$), and nights 6 through post-drug 2 ($F=17.96$; $df=3,27;p<.01$). As such, comparisions are to baseline values. Saline does not significantly alter PS.

Table 28 lists the frequency of wake episodes. Carbachol and saline do not alter wake frequency. Scopolamine, decreases wake episodes during the period of drug infusion (Chi-square=16.29;df=3;$p<.01$). Table 29 lists the frequency of SWS episodes. Scopolamine reduces the number of SWS episodes during the period of drug administration (Chi-square=16.13;df=3;$p<.01$). Table 30 summarizes the frequency of PS. Scopolamine has no effect. Carbachol increases PS frequency during nights 2 through 5 but this is just

short of being statistically significant (Chi-square=6.73;df=3;p<.1). Saline alters PS frequency but this is at the post-experimental baseline level (Chi-square=16.52; df=3; p<.01).

Tables 31 and 32 summarize the mean duration of wake and SWS. There is no alteration in wake length as a function of drug or saine. Scopolamine increases the length of SWS episodes during nights 2 through 5 only (F=11.34;df=3;p<.05). Saline and carbachol have no effect. Table 33 summarizes the mean PS duration. Scopolamine and saline have no effects. Carbachol, however, significantly reduces the length of PS episodes during nights 2 through 5 (F=9.15;df=3,27;p<.05).

The first 24 hours of pump operation

Tables 11, 12, and 13 summarize the percent TST, SWS and PS during the day and night cycle. During the hours following puump implant, carbachol and scopolamine animals experience a significant decrease in TST. Saline animals experience a 17% decrease which is not significant. During the night cycle the three groups show an increase in TST, but this is not significant. With respect to SWS, only carbachol and scopolamine animals experience a significant decrease during the day cycle. All three groups indergo significant reductions in PS during the day hours following surgery. During the night cycle there is no significant change in PS. However, carbachol and scopolamine animals continue to experience a PS loss while saline animals show a 10% increase.

INTER-SITE COMPARISIONS

24 hr cycle

Figure 19 summarizes the percent PS deviation from pooled baseline using carbachol. There is no significant difference between the pontine and the midline ponto-medullary group. However, the pontine group is significantly different from the left-side ponto-medullary group ($t=2.73$; $df=7$; $p<.05$), and the ventricular group ($t=2.73$; $df=7$; $p<.05$). There are no significant differences between the other three groups.

Figure 20 summarizes the percent PS deviation from pooled baseline as a function of scopolamine infusion. There are no significant differences between the three sites.

Figure 21 summarizes the percent PS deviation from pooled baseline with saline. There are no differences.

Day Cycle

Figures 22, 23, and 24 summarize the percent PS deviation from pooled baseline using carbachol, scopolamine and saline. Between site comparisions reveal no differences.

Night Cycle

Figure 25 summarizes the percent PS deviation from pooled baseline using carbachol. There is no difference between the pontine and the midline ponto- medullary group. However, the midline pontine group is significantly different from the left-side ponto-medullary group ($t=3.21$; $df=8$; $p<.05$), and the ventricular group ($t=3.52$; $df=7$; $p<.05$). There are no significant differences between the other three groups.

Figures 26 and 27 summarize the percent PS deviation from pooled

baseline using scopolamine and saline. There are no differences in each category.

Experiment 1: Discussion

The results of this experiment support the hypothesis that long-term infusions of cholinergic agents into the brainstem produce site specific, selective long-term alterations in PS. More specifically, it is found that infusions of carbachol, an acetylcholine agonist, increases PS, while scopolamine, a muscarinic blocker, has the opposite effect.

An Alzet osmotic minipump is used to infuse carbachol, scopolamine or saline into three brainstem sites, viz., (a) pontine nuclei, (b) ponto-medullary nuclei, and (c) the fourth ventricle. The salient feature of this method is that it enables the drug to be delivered at a constant rate over a period of seven days.

The results show that infusion of carbachol into the pontine brainstem produces a selective increase in PS. The almost 90% increase in PS occurs during the first five days of drug infusion and is seen primarily during the night cycle. No changes in total sleep time or SWS are detected. The PS augmentation is due to an almost 130% increase in PS frequency and not the result of an increase in PS duration.

In order to assess the effects of diffusion of carbachol to neighboring nuclei, infusion of carbachol into other brainstem areas such as the midline pontomedullary, left-side ponto-medullary, or the fourth ventricle was performed. These sites fail to produce alterations in PS. Since no alteration in PS occur we presume that carbachol did not diffuse into areas remote from the intended target.

We therefore conclude that the effect of carbachol is site specific. However, an alternative possibility is that the cannula may have destroyed cholinoceptive PS neurons, thereby impairing the PS generating mechanisms. This interpretation, however, seems unlikely because disruption of these sites does not disrupt the normal production of PS. It is clear that the optimum location of the cannula is in the midline pontine area, such that the cholinoceptive reticularis pontis caudalis neurons on either side of the cannula may be influenced by the pharmacological agents. Indeed, this area has long been suspected to produce PS (Jouvet, 1972; Hobson and McCarley, 1977).

The PS increase induced by carbachol in the pontine groups is not a PS rebound. A rebound might be expected considering that during the first nine hours following pump implant the animals demonstrate an 85% PS loss. However, all animals undergo significant PS loss at this time (table 13), but only the pontine animals display a PS increase during the subsequent days. The scopolamine animals, on the other hand, continue to experience a PS loss throughout the experiment. Additionally, once the pumps have exuded their contents, PS returns to baseline levels (Fig. 4). In short, the changes seen in the pontine carbachol and scopolamine groups must be drug related. Carbachol produces a site-specific PS increase. Scopolamine, on the other hand, has an inhibitory effect on PS, which is not site-specific. The PS decrease is seen shortly after pump implant and it persists even after the pumps have ceased to operate(Figs. 8, 13, 17). In this regard, the effects of scopolamine on PS are

significantly longer than carbachol. The scopolamine effects occur during the day cycle, and the PS decrease can be accounted for by both a reduction in PS frequency and duration. The divergent alterations in PS produced by these two pharmacological agents is summarized in Fig 31.

Possible mechanisms of action

(1) The contrasting effects that we find in this study are predicted by the reciprocal-interaction model (Hobson and McCarley, 1977) which posits that alterations in FTG neuronal excitability might generate PS. Moreover, the nature of the reciprocal interaction suggests that carbachol and scopolamine might alter PS at opposite times in the 24 hr sleep rhythm; our results provide evidence for the correctness of the model. For instance, carbachol increases PS during the night cycle, a period when the animal is awake and PS is least likely to occur. No effects can be discerned during the day cycle, perhaps because the PS generating machinery is operating at its genetically determined peak rates; carbachol failing to drive the cycle faster. During the night, however,the effects of carbachol may serve to lower pontine neuronal firing thresholds so that the PS episode is more likely to be triggered. Indeed, the data lend support to this interpretation since in the night cycle there is a 130% increase in PS frequency. Additionally, an examination of the 24 hr sleep profile (Fig. 4) reveals that carbachol serves to retard the PS decrease during the night cycle.

Scopolamine, on the other hand, serves to decrease PS during the day cycle, a period of maximum PS generation, and does so by

decreasing PS frequency and duration. One possible explanation for this effect is that scopolamine exerts a general hyperpolarizing effect on the pontine neurons, thereby reducing the likelihood of the PS episodes to occur. In the event that PS is triggered, the episodes are aborted because of a lack of sustained underlying neuronal activity.

Neuropharmacological evidence lends support to our interpretation that carbachol and scopolamine exert such neuronal destabilizing effects. For instance, it is known that both of these pharmacological agents affect muscarinic receptors and that the excitation is due to a shift in membrane potential (Goodman and Gillman, 1980). However, instead of the depolarization being associated with a fall in membrane resistance, as is normally the case, the excitation is due to an increase in membrane resistance to potassium (Woody etal., 1974). The net effect of this is that a relatively slow excitatory post-synaptic potential, with a time period of 5 secs- 3 minutes, is produced (Krnjevic and Phillis, 1963). Krnjevic (1974) notes that in this mode, muscarinic stimulation has a priming effect which can set the level of responsivity of neurons, either individually, or in groups, depending upon the interconnectivity of the neurons.

(2) Secondly, evidence has been provided suggesting that cyclic guanosine monophosphate (cGMP) mediates muscarinic receptor sensitivity. For example, acetylcholine causes an increase in tissue content of cGMP, and this increase is evoked only by muscarinic agonists and prevented by atropine, a muscarinic blocker (George etal., 1970; Lee etal., 1972; Eichorn, 1974). In the cortex, Phillis

(1974) has found that cGMP induces excitation, while Stone etal., (1975) have shown that Ach and cGMP, iontophoresed extracellularly, increase the firing rate of pyramidal tract neurons. Swartz and Woody (1976) have shown that these effects can be antagonized by atropine. These observations suggest that cGMP acts as a second messenger for muscarinic neural transmission.

While cGMP may mediate membrane excitability levels, it is not clear how receptor sensitivity is altered to produce regular PS rhythmicity. In other words, if PS is due to increased discharge of a group or groups of pontine neurons, how are the pontine receptors activated?

It is suggested that protein synthesis might be responsible for modulating receptor activation (McGinty and Drucker-Colin, 1982). In support of this view, there is evidence to indicate that protein synthesis inhibitors decrease PS without affecting SWS, and without any compensatory PS rebound (Gutwein, Shiromani and Fishbein, 1979; for review see Drucker-Colin, 1979). More importantly, it has been found that protein synthesis inhibition decreases the frequency of PS episodes (McGinty andDrucker-Colin , 1979); this decrease might be due to overall attenuation in spike discharge of pontine reticular units (Drucker-Colin etal., 1982). In the latter study, a push-pull cannula system was used to infuse chloramphenicol, a powerful inhibitor of synaptic plasma membrane protein synthesis, into the pontine reticular formation. The results showed that the drug, in comparision to Ringer's solution, significantly reduced pontine unit activity and produced a selective decrease in PS. Moreover, during the period of

drug perfusion, there was an increased incidence of aborted PS episodes. The authors concluded that the chloramphenocol prevented the pontine neurons from achieving a minimum discharge rate necessary for PS generation.

The results of the present experiment support such a conclusion. Carbachol, by repeatedly stimulating muscarinic receptors at a time when receptor activity is at a minimum, reduces discharge threshold and thereby PS is more likely to be triggered. Scopolamine, on the other hand, blocks pontine receptor activity and prevents PS generation.

Our results therefore fit nicely with the reciprocal-interaction model in that cholinergic manipulation of pontine neurons produce concomitant alteration in PS. The results also suggest that activation of muscarinic receptors serves to produce changes in PS and that overall PS rhythmicity might be under the control of a protein cycle which periodically activates a dormant muscarinic receptor. Discerning the nature of the receptor activation and the mechanisms of action of the cholinergic modulating drugs employed in this study will have to await further research.

EXPERIMENT 2

Introduction

The purpose of this experiment is to increase the drug infusion time from 7 to 14 days, thereby determining whether PS can be augmented for longer periods.

Subjects

Five male Holtzman rats are used. The animals are handled and housed as previously described.

Procedure

The method of electrode, cannula and pump implant is as previously outlined. In this study, however, a 14 day Alzet mini-pump (model 2002) filled with carbachol (0.5 ug/hr) is used.

One week after electrode and cannula implant, the animals are connected to the recording cables and allowed 2 days of adaptation. On day 3, at 9 AM, a 24 hr baseline record is obtained. At 9 AM, the following morning, the pump is implanted and the animals reconnected to the recording cables. However at this time no EEG recording is obtained. The polygraph is switched on the next morning at 7 AM and a 24 hr record obtained. Thereafter, a 24 hr record is obtained every alternate day until day 14 when three consecutive 24 EEG records are obtained. Two weeks after the pumps have ceased operating a 24 hr post- experimental record is obtained. Subsequently the animals are sacrificed and the brains removed for histological verification of cannula placement.

The method of data analysis is as previously described.

RESULTS: EXPERIMENT 2

The cannula placement is schematized in Fig. 28.

24 hour cycle

One-way repeated measures ANOVA's with Scheffe post-hoc comparisions are used. Table 34 summarizes the percent TST, SWS and PS. There are no significant differences across days.

Day Cycle

The 24 hr sleep cycle is partitioned into DAY-NIGHT blocks. A one-way repeated ANOVA with Scheffe tests is used to analyze changes in TST, SWS and PS across the day cycle. Table 35 summarizes the percent TST. Scheffe comparisions between baseline and post-experimental baseline reveal no significant difference. Moreover, pooled baseline is not significantly different from the second day of pump operation, days 4 through 10, and days 12 through postdrug 2. Table 35 also summarizes the percent SWS. There are no significant differences.

Figure 29 summarizes the percent PS deviation from pooled baseline. There are no significant changes during day 2 of carbachol infusion. However, commencing on day 4 there is a significant increase in PS. Scheffe comparision reveals that days 4 through 10 show a significant 55% increase over pooled baseline ($F=30.04$; $df=10,40$; $p<.05$). On days 12 through postdrug 2, PS levels continue to be 46% over pooled baseline values ($F=21.0$; $df=10,40$; $p<.05$).

Table 36 summarizes the frequency of wake, SWS and PS episodes. Chisquare tests reveal no significant change in wake frequency. SWS

episodes, on the other hand, show a slight increase (Chisquare=10.24; df=4; $p<.05$). PS frequency increases significantly by 100% during the first 10 days of carbachol infusion and thereafter begins to assume baseline levels (Chisquare=9.67; df=4; $p<.05$).

Table 37 summarizes the mean duration of wake, SWS and PS. Dunnett's test reveals that the mean duration of wake episodes during postexperimental baseline is significantly higher than baseline; the other days are not significantly different. With respect to SWS duration, days 2, 4 and postdrug 1 show significantly shorter SWS lengths while postexperimental baseline has longer duration of SWS than baseline. The mean duration of PS is unchanged, except on day 2 when it is significantly shorter than baseline.

Night Cycle

Table 35 summarizes the percent TST and SWS. There are no changes. Figure 35 summarizes the percent PS deviation from pooled baseline. Even though there is a decrease in PS across the night cycle, it is not significantly different from pooled baseline.

Table 36 and 37 summarize the frequency and mean duration of wake, SWS and PS. There are no changes in frequency of wake, SWS and PS episodes. Moreover, the mean duration of wake and PS is unchanges. SWS mean length, on the other hand, undergoes a significant decrease during nights 4, 6, and 10 through postdrug 2.

Experiment 2: Discussion

The results of experiment 1 indicate that seven day continuous infusion of carbachol into the pontine area increases PS during the first five days of pump operation; the present experiment examines whether the effects can be extended beyond this period. The results indicate that infusion of carbachol for 14 days produces a selective increases in PS lasting the duration of pump operation.

This experiment differs from experiment 1 in several ways. First, the site of drug infusion is caudal to the genu of the seventh nerve; this area is identical to the level of the ponto-medullary area in experiment 1. Second, the pumps employed in this experiment are different; the volume of solution delivered to the target area is half that of experiment 1, although the amount of carbachol delivered per hour is identical in both experiments.

The results show selective alterations in PS. The PS levels measured on the second day of pump operation are similar to pooled baseline levels; commencing on day four of drug infusion there is a selective PS increase which persists for the duration of pump operation (ie., postdrug 2). These findings are summarized in Fig 29. The PS increase occurs during the day cycle and is due to an increase in frequency of PS episodes. There is no change in TST or SWS.

In contrast to experiment 1, the PS increase induced by carbachol occurs during the day cycle. This PS increase during the day cycle is not completely consistent with the argument put forward in experiment 1 regarding the ineffectiveness of carbachol to induce a PS increase

during the day cycle.

One possible explanation of this finding is that the day time PS increase might be a rebound due to a PS decrease seen during the night cycle, particularly the 1-7 AM period. The PS levels during this part of the night cycle are significantly lower then baseline values ($t=5.07$; $df=9$; $p<.01$), serving to reinforce the possibility that the PS increase during the day cycle might be an artifactual PS rebound. In experiment 1, no such decrease is observed, strengthening our belief that the PS increase seen there is drug related. Yet, despite the decrease in PS during the night cycle, the absolute time loss is small relative to the enormous increase seen during the day cycle. Generally in previous reports of PS rebound experiments, at best the rebound is no more then a one for one replacement, whereas in the present experiment the day cycle increase is 2 fold greater then the PS decrease seen during the night cycle. Perhaps the PS decrease serves to sensitize muscarinic receptors so that carbachol exaggerates the PS rebound. Similar sensitization of catecholamine receptors has been demonstrated following PS deprivation (Moginicka, 1981). Moreover, in humans, pre-treated with scopolamine, administration of arecoline exaggerates a PS increase (Sitaram etal., 1979). The latter study indicates that scopolamine, a proven PS suppressor, sensitizes muscarinic receptors. Perhaps carbachol serves to exaggerate the PS rebound and in a further study this might be tested by administering carbachol to PS deprived animals. In sum, it is suggested that PS deprivation might serve to sensitize muscarinic receptors such that a subsequent cholinergic excitation might produce an exaggerated PS rebound.

EXPERIMENT 3

Introduction

Converging evidence from a variety of studies suggests that a cholinergic component of the ascending reticular activating system may play a vital role in information processing. There is also evidence to indicate that perhaps activation of this system during PS serves to facilitate memory consolidation. To test the latter possibility, this experiment determines whether long-term infusions of cholinergic agents into areas postulated to trigger PS alters the durability of a memory trace.

Subjects

48 male Holtzman rats weighing 400 gms are used. The animals are housed singly in wire mesh rat cages with food and water available freely. Constant temperature and a 12 hr light-dark cycle are maintained.

Experiment design

The animals are implanted with the cannula and randomly assigned to one of six groups (N=8/group): (i) carbachol - 7 day test, (ii) carbachol - 21 day test, (iii) scopolamine - 7 day test, (iv) scopolamine - 21 day test, (v) saline - 7 day test, and (vi) saline - 21 day test.

The drug dosage and saline concentration are as previously stated in experiment 1.

Apparatus

A two-way active avoidance shuttlebox is used. The shuttlebox is

constructed of 1/4 inch white opaque Plexiglas with a stainless steel grid floor. The box is divided into two compartments by a constriction in the center which leaves a passageway approximately 4 inches wide.

The shuttlebox is connected to an eight grid fully automated shock scrambler which evenly electrifies the floor of either compartment during any trial. Regulation and control of the intensity and duration of shock, and the duration of each trial is pre-programmed with logic circuitry by the experimenter.

A light (25 W) and buzzer (Potter and Brumfield, 6 V) suspended over the center of the box serves as the conditioned stimulus (CS). The duration of CS is under control of the logic circuitry also.

Procedure

The animals are anesthetized with Nembutal and an L-shaped cannula prepared as previously described, is lowered into the brainstem. Two stainless steel screws implanted anterior to lambda and on either side of the mid-sagittal sinus serve as anchors. The screws and the cannula are secured to the skull with dental cement.

One week after cannula implant, at 8 AM, the Alzet pump is implanted. Seven hours after pump implant, the animals are placed inside one compartment of the shuttlebox and allowed one minute of adaptaion to the test apparatus. Subsequent to this the animals are given 50 consecutive training trials with an inter-trial interval of 30 secs. Each trial consists of the classical conditioning paradigm of conditioned stimulus (5 secs of light ad buzzer) followed immediately by the unconditioned stumulus (foot shock, 0.2 - 0.3 mA).

A timer is started automatically with each trial and is stopped manually when the animals cross into the opposite compartment. If the animals cross over during the time that the CS is on the time is noted indicating that the animal avoided the shock. If the animals fail to cross over withing the 5 secs then the foot shock is automatically presented. The shock is terminated when the rats escape into the opposite compartment. The time taken to escape the shock is also noted.

Immediately following the last trial the animals are placed back in their home cages. Before the start of the next training session, the shuttlebox is scrubbed down with acetone so as to eliminate all odor cues.

Either 7 or 21 days after training the animals are returned to the shuttlebox and tested for retention of the avoidance behavior. The testing procedure is identical to that during training. Additionally, the time of testing is same as training.

Immediately following the testing session, the animals are sacrificed by administering a lethal dose of Nembutal.

Data analysis

The percent avoidance score for each learning series is computed using the following equation: Percent avoidance= (# avoidance/# trials) X 100.

Group percent avoidance scores are computed for all 50 trials during training and testing. A repeated measures ANOVA is used to test for differences between training and testing.

To gain better understanding of acquisition and retention of the

task, the percent avoidance scores during the last 5 and first 5 trials of training and testing are computed for each group. A repeated measures ANOVA is also used in this instance.

RESULTS: EXPERIMENT 3

Two-way repeated measures ANOVA's and twoway randomized block ANOVA's, both with tests of simple effects, are used to test for significance between training and testing scores, and for differences between drug conditions.

Table 38 summarizes the percent avoidance scores for all 50 trials. There are no significant differences between groups during training and testing. A repeated measures ANOVA reveals that all animal groups treated with either carbachol, scopolamine or saline show significant increases in percent avoidance during testing.

Figure 30 summarizes the percent deviation of the avoidance scores during the last and first five trials of training and testing sessions. It is reasoned that by partitioning the avoidance scores in such a manner, a much clearer understanding of memory may be gained. There are no significant differences between drug and saline conditions during training and testing. However, comparisions of percent avoidance scores at the 7 day testing session reveals that the animals in the scopolamine condition demonstrate a significant 44% decline in retention of shuttlebox learning. It must be noted, though, that all groups show a retention deficit, but only the group administered scopolamine shows a statistically significant deficit. Moreover, the retention deficits in all groups at the 7 day testing session are much more marked than those at the 21 day testing session.

Experiment 3: Discussion

This experiment tests the general hypothesis that activation of brainstem neuronal pathways during PS serves to produce a state conducive to memory storage. More specifically our working hypothesis is that infusion of carbachol would attenuate decay of a learned habit measured 7 or 21 days after training, while scopolamine would augment the forgetting process. The results, however, are inconsistent and in certain ways difficult to interpret.

Overall examination of the total testing session avoidance scores indicate that at the seven or twenty-one day interval all three groups are equally able to relearn the two-way shuttlebox task. In order to more carefully assess whether memorial processes are effected by the drug infusion treatments, the data are analysed for difference between the last five and first five trials of the training-testing sessions; the last five trials of the training session representing a clearer measure of the actual acquisition of the task, while retention (memory) of the learned response is best assessed by examining the first five trials of the testing session. Figure 30 summarizes this analysis.

At the 7 day train-test interval, scopolamine treated animals demonstrate a significant memory loss in comparision to saline and carbachol treated animals. At the 21 day train-test interval, on the other hand, all animals show good retention of the task.

The finding that the scopolamine animals show a memory loss at the

7 day point and not at the 21 day session gains importance particularly in light of the finding that this drug produces a selective PS loss for 9 consecutive days. The memory loss at the 7 day train-test interval may be a retrieval failure, perhaps due to scopolamine induced alterations in neuronal networks subserving both PS and memory. A similar retrieval failure has been demonstrated by Quartermain and Botwinick (1975). They show that mice treated with cyclohexamide, a protein synthesis inhibitor, prior to training, demonstrate a significant memory loss 24 hrs after training but not 48 hrs later. Additionally, they show that administration of catecholamine agonists, pheniprazine and pargyline, prior to retention testing reverses the amnesia. It is useful to note that protein synthesis inhibitors and catecholamines also produce alterations in PS.

Another explanation for the memory loss at the 7 day interval is that it is a state- dependent performance loss due to the continued effect of scopolamine on the CNS. This is strengthened by the fact that at the time of testing at 7 days, scopolamine animals, in contrast to carbachol or saline animals, demonstrate a significant PS decrease. Additionally, even the carbachol and saline animals may be demonstrating a state-dependent effect since in Fig. 30 it is seen that these groups show an avoidance decrement at the 7 day interval. However, this decrement is not significant. In sum, while it is tempting to speculate that the poor scores seen in the scopolamine animals may be due to alterations in retrieval processes, a state-dependent effect cannot be ruled out.

The results of this experiment, therefore, suggest the possibility that chronic infusions of cholinergic agents into the brainstem may alter neuronal excitability which in turn may influence memory storage processes. Considering that both carbachol and scopolamine induce long-term changes in PS, it would be possible to demonstrate underlying memory related changes in neuronal excitability by applying ECS in a time dependent manner following pump implant. It is predicted that ECS given after carbachol infusion will fail to alter memory since the carbachol may shorten the lability of the memory trace. There is evidence to indicate that ECS administered after a stimulant fails to produce retrograde amnesia (Hunter etal., 1977). With respect to scopolamine, it is predicted that ECS would induce amnesia when given as much as nine days following pump implant; the nine day period is based upon the extent of PS deprivation induced by scopolamine in experiment 1.

In conclusion, there is extensive evidence to indicate that the neuronal pathways involved in memory storage might be influenced by the activation of a cholinergic system during PS. There is also evidence to suggest that changes in cholinergic activity might produce alterations in both PS and memory. In an attempt to confirm these hypotheses, carbachol, scopolamine or saline were infused into the brainstem. The findings do not reveal any alteration in retention of a shuttle-box response as measured 21 days following acquisition, although scopolamine may have produced a retrieval failure at 7 days.. The findings, however, are not conclusive. It is suggested that a more potent amnesic agent (eg., ECS) administered in a time dependent

manner may serve to elucidate changes in behavioral amd memory processes induced by chronic carbachol or scopolamine infusions.

General Discussion

Ever since the discovery of PS, scientists have attempted to determine how it is generated, and speculated as to its biological significance. While neither of these goals have been attained, researchers have provided important clues as to our understanding of the brain regions and neurotransmitter systems critically involved in PS generation and maintenance. Presently, the task facing the sleep researcher is determining the interaction between neurotransmitters and brain nuclei in promoting a condition suitable for PS generation, and in describing the mechanism responsible for regular PS rhythmicity.

The purpose of this dissertation, therefore, is to add further to this body of research and to specifically determine whether long-term infusion of cholinergic agents into brainstem regions, postulated to play a critical role in PS, produce prolonged alterations in PS. We consider this study to be particularly important since before a neural center can be designated a PS trigger area, it is necessary to demonstrate that the center can produce divergent responses to opposing neurotransmitter modulating agents over a prolonged time period. Secondly, the study provides important information regarding the nature of PS generation and PS rhythm as a whole.

In experiment 1, carbachol, scopolamine or saline are infused for seven days into either the pontine brainstem, ponto-medullary area or the fourth ventricle. The rationale for infusing the agents into several areas is to delineate anatomical specificity, and to determine

if drug diffusions confound the findings.

The results show that in the pontine group carbachol infusion produces a selective increase in PS during the first five days of pump operation. The increase occurs during the night cycle and is due primarily to an increase in the frequency of PS episodes. Scopolamine, on the other hand, decreases PS for nine days during the day cycle, and it does so by reducing PS frequency and duration. In contrast to carbachol, the effect of scopolamine is not site specific and may even be obtained with infusions into the fourth ventricle. In this respect it is difficult to delineate whether the PS decrease, obtained in the groups with cannula in areas outside the pons, is due to drug diffusion into the pons or whether ponto-medullary muscarinic receptors and those receptors lining the fourth ventricle are genuinely involved in modulating pontine firing. While the carbachol effect clearly indicates that the action of the drug is localized, scopolamine seems to diffuse widely. Another possibility is that scopolamine may be a more potent compound. Certainly a dose response study would be necessary in order to elucidate the effects of scopolamine on specific brainstem sites. Figure 31 summarizes the divergent effects produced by these two agents.

These results are generally consistent with those reported by Vivaldi etal (1980) and others (Baxter, 1969; George etal., 1964) that carbachol augments paradoxical sleep, although in the present research in contrast to the previous work the effects are due to an increase in PS frequency rather than duration. The difference may be due to the fact that in the present study the drug was chronically infused with

twenty-four hour continuous monitoring of the subjects sleep-wake cycle. The results also confirm the findings of Sitaram etal., (1976, 1977, 1978) in which systemic injections of physostigmine or arecoline increase PS in humans.

An important finding in the human studies was that cholinergic treatment decreased the latency to the PS episode. This suggests that the cholinergic system may be involved in PS periodicity (ie., trigger generation) rather than PS duration; a finding which is consistent with the present results. In the present work it is not possible to obtain a measure of the latency to PS onset because of the variability associated with recovery from the trauma associated with the pump implant. Nevertheless, it can be inferred from the increased frequency of PS cyclicity tnat the mode of action of carbachol is to speed up the sleep- waking cycle. Additionally, the changes in PS occur primarily during the night cycle. Scopolamine, on the other hand, slows down the PS cycle since there is a 50% attenuation in PS frequency and duration.

The alterations in PS, therefore, provide important clues regarding the nature of the cholinergic mechanisms and how they might be involved in PS generation. It is known that carbachol and scopolamine both effect muscarinic receptors, and these receptors have been shown to exist in the brainstem. For instance, Krnjevic (1974) notes that muscarinic receptor stimulation is a result of a slow EPSP which might be mediated by cGMP. It is our hypothesis that a slow depolarization by cholinergic stimulation might prime pontine neurons; any additional synaptic input would therefore trigger a general

excitation through the neuronal chain. Consistent with this hypothesis is the finding that there is a steady increase in pontine firing rates during the transition period from SWS to PS (Hobson and McCarley, 1977). Our hypothesis is that carbachol stimulates the pontine muscarinic receptors, thereby producing a generalized depolarization and a subsequent PS increase. During the day cycle, carbachol is unable to alter PS because at this time muscarinic activation is already at maximum. During the night, however, there is a gradual reduction in receptor sensitivity which is reversed by carbachol. On the other hand, the effects of scopolamine are seen during the day cycle when the drug inhibits receptor activity thereby preventing the generalized excitation needed for PS. The fact that scopolamine does not totally abolish PS suggests that the treatment is not complete. The finding that scopolamine shortens the duration of the PS episodes in addition to its effects on frequency would suggest that the PS episode is aborted because of a lack of sustained underlying neuronal activity.

Since neuronal membrane permeability is determined by receptor activity, it is important to determine the mechanisms which modulate receptor sensitivity. McGinty and Drucker-Colin (1982) suggest that increased protein synthesis activates a dormant receptor which then primes the neuron for excitation. Considerable evidence exists to support this, and particularly interesting is the study by Drucker-Colin et al., (1975) who showed that the concentration of proteins in perfusates obtained from the medial reticular formation was higher during PS. More importantly it is seen that

chloramphenicol administered using a push-pull system lowers the unit activity of pontine neurons and increases aborted PS episodes (Drucker-Colin etal., 1982). They speculate that chloramphenicol may prevent the activation of the receptor thereby suppressing pontine firing. The divergent alterations in PS induced by scopolamine and carbachol in experiment 1 are consistent with such a conclusion.

As part of this discussion it is, of course, necessary to assess the effects of chronic infusions of pharmacological agents on receptor activity. Current evidence (Friedhoff and Alpert, 1978) indicates that receptor sensitivity is governed by availability of neurotransmitter and that any change in the supply of the neurotransmitter will provoke the receptor to achieve homeostasis. In other words, receptor blockers will sensitize the receptor while agonists will desensitize it. Since chronic infusion of cholinergic agonists and antagonists is being carried out in this research, it is reasonable to assume that these compounds may be operating by way of altering receptor sensitivity, and that changes in PS might reflect attempts by the receptors to achieve homeostasis. For example, it would be appropriate to predict that chronic carbachol infusions will initially stimulate PS increases, but as the receptors are desensitized the PS levels will return to baseline.

The results support this prediction (Fig. 10) since, even though the drug is infusing for seven days, the PS increase is seen during the first five days of pump operation only, with a linear decline in PS augmentation as each day passes.

The scopolamine induced PS alterations, in contrast to carbachol,

persists for nine days following pump implant. It is possible that the PS decrease persists longer than nine days, but in this study the EEG recording was terminated at the nine day point. An important further study would be to infuse scopolamine for 14 days using the longer lasting pumps. Such an experiment will provide important data considering receptor attempts to normalize PS levels. Nevertheless, the results, as illustrated in Figures 8, 13 and 17, reveal that receptor sensitization is occurring in the scopolamine treated animals; after an initial 70% PS loss there is a trend towards restoring PS to baseline levels. It is important to note that during post-experimental baseline, which is 14 days after the pumps have ceased operating , PS levels return to baseline levels.

In addition to alterations in receptor sensitivity, chronic cholinergic infusions might also alter catecholamine and indoleamine levels. While such interactions have been reliably demonstrated in the forebrain (Pradhan and Bose, 1978), one possible interaction that might occur in the brainstem would be the noradrenergic- cholinergic interplay postulated by Hobson and McCarley (1977). One can only speculate that the anatomical pathway producing the PS alteration is similar to the ascending cholinergic system of Lewis and Shute (1967). However, given the interactions between neurotransmitter systems, the involvement of the noradrenergic or 5-HT pathways cannot be ruled out.

Summary and Conclusions

Experiment 1 provides important data indicating that infusions of carbachol or scopolamine into the pontine region, an area long

postulated to play a critical role in PS generation, produces significant PS changes. It is suggested that the generation of PS is influenced by alterations in muscarinic sensitivity: cholinergic agonists serving to depolarize cells in the PS generation area while a receptor blocker (antagonists) inhibits or hyperpolarizes cells in the PS generating area leading to reduction of PS.

In experiment 1 carbachol was infused for 7 days, in experiment 2 the period of drug infusion was doubled with the expectation of gaining insight into the number of days that PS may be increased. The results, however, are inconclusive in that the PS alterations seen during the day cycle might be a PS rebound caused by a significant PS loss during the night cycle. This possibility is strengthened by the fact that the area of carbachol infusion in this experiment can be compared to experiment 1, where two groups with similar cannula placements failed to show any changes in PS. Also, it is reasonable to assume that receptor desensitization would normalize the PS levels fairly quickly. Yet, the PS increase persists for 16 days after pump implant with only a slight diminution during days 10 through postdrug 2. These factors would suggest that the results of experiment 2 may be confounded by a PS loss occurring during the night cycle.

In addition to investigating the alterations in PS, this research also assessed the effects of long-term infusions of cholinergic agents on memory processes. In experiment 3 the hypothesis tested was that activation of neuronal brainstem pathways during PS establishes a state conducive to memory storage. Chronic carbachol, scopolamine or saline infusions were made in independent groups of rats. After

training in a shuttle-box, the animals were tested for retention of the conditioned response either 7 or 21 days following training. The results showed that at 7 days all treatment groups display a decline in retention. In the scopolamine group the retention failure was found to be statistically significant; at 21 days, however, all treatemnt groups are equivocal.

The results are difficult to interpret because of the failure of the control (saline) group to show a normal degree of forgetting at 21 days, possible because the shuttle-box training was excessive and/or the task was too easily learned. Thus the failure of the scopolamine group to show a retention deficit can be explained by leaning to the already existing broad literature showing that even very powerful amnestic agents fail to disrupt firmly ingrained memory.

The scopolamine group, in contrast to the carbachol or saline groups, showed significant memory loss at the 7 day interval. This finding coupled with the fact that scopolamine reduces PS for 9 days suggests that this drug may produce a deficit in memory retrieval at the 7 day point. However, it is also possible that the deficit might be an artifact due to the continued effect of scopolamine on the CNS.

The inability to draw clear meaning from this experiment does not preclude the possibility that scopolamine and carbachol might alter the period of memory lability. It is possible that scopolamine by disrupting the neuronal mechanisms involved in PS extends memory lability. Carbachol, on the other hand, by inducing depolarization and thereby PS, might be shortening the labile period. This hypothesis might be tested by administering ECS in a time dependent manner

following pump implant and training.

The results of this dissertation implicate the cholinergic system in PS generation and memory, and suggest that alterations in brainstem cholinergic activity might produce alteration in both PS and memory. Indeed, cholinergic dysfunctions associated with aging appears to influence memory (Bartus etal., 1982) and PS (Prinz and Rasskind, 1978). Moreover, a cholinergic disturbance might be a participant in the sleep disorders associated with primary depression (Sitaram etal., 1980). The results of this dissertation therefore provide further evidence of the important role of the cholinergic system in memory and sleep.

Briefly then, the present work has examined the role of the cholinergic system in PS and memory. The results show that chronic infusions of a cholinergic agonist into the pontine brainstem selectively increases PS during the night cycle, while scopolamine decreases PS during the day cycle. It is suggested that these effects are due to alterations in muscarinic receptor activity, and that in normal animals, regular PS rhythmicity might be due to a protein controlled modulation of receptor activity.

TABLE 1

Summary of percent TST (±SEM) over 24 hours

24 hr cycle

	Baseline + Post-experimental	2-5	6-Post-drug 2
Carbachol (n=5)	50.1 (2.4)	51.9 (2.9)	47.5 (2.2)
Scopolamine (n=5)	55.0 (2.5)	55.8 (2.0)	52.5 (1.9)
Saline (n=5)	55.7 (1.3)	58.6 (2.4)	57.4 (2.7)

TABLE 2

Summary of SWS of total time (±SEM) over 24 hours

24 hr cycle

	Baseline + Post-experimental	2 - 5	6--Post-drug 2
Carbachol (n= 5)	44.3 (2.0)	44.1 (2.4)	40.2 (1.8)
Scopolamine (n= 5)	47.1 (2.2)	50.8 (2.1)	47.8 (1.8)
Saline (n=5)	47.7 (1.1)	50.1 (2.4)	49.2 (2.4)

TABLE 3

Summary of percent TSI (±SEM) during the day and night cycle

	Baseline + Post-experimental	2-5	6 -Post-drug 2
Day Cycle			
Carbachol (n= 5)	66.0 (4.6)	63.7 (3.4)	63.2 (2.7)
Scopolamine (n= 5)	66.3 (4.4)	66.9 (3.2)	68.7 (3.6)
Saline (n= 5)	71.5 (1.8)	71.9 (3.7)	72.5 (2.1)
Night Cycle			
Carbachol (n= 5)	34.8 (1.5)	40.3 (3.9)	30.4 (3.2)
Scopolamine (n= 5)	43.8 (4.3)	42.3 (4.1)	35.7 (4.0)
Saline (n=5)	40.3 (2.1)	45.4 (3.2)	40.9 (4.0)

TABLE 4

Summary of percent SWS of total time (±SEM) during the day and night

Day Cycle

	Baseline + Post-experimental	2-5	6-Post-drug 2
Carbachol (n= 5)	57.4 (3.7)	53.9 (2.3)	53.6 (1.9)
Scopolamine (n= 5)	57.0 (3.4)	63.4 (3.1)	63.1 (3.5)
Saline (n= 5)	60.5 (1.7)	60.4 (3.6)	61.1 (2.2)

Night Cycle

Carbachol (n= 5)	31.6 (1.4)	34.3 (3.6)	27.2 (2.9)
Scopolamine (n= 5)	37.2 (3.1)	38.2 (3.9)	32.0 (3.6)
Saline (n= 5)	35.3 (1.3)	39.8 (2.3)	36.1 (3.1)

Table 5

Average frequency of W episodes during the day and night cycle

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=5)	95	109	93	80
Scopolamine (n=5)	66	57	55	60
Saline (n=5)	63	59	59	61

Night cycle

Carbachol (n=5)	92	101	77	85
Scopolamine (n=5)	71	49	51	63
Saline (n=5)	64	70	63	70

Table 6

Average frequency of SWS episodes during the day and night cycle

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=5)	102	120	104	92
Scopolamine (n=5)	88	72	74	84
Saline (n=5)	93	90	87	90

Night cycle

Carbachol (n=5)	89	103	76	85
Scopolamine (n=5)	81	58	55	78
Saline (n=5)	72	85	71	81

Table 7

Average frequency of PS episodes during the day and night cycle

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=5)	33	45	44	31
Scopolamine (n=5)	34	20	28	38
Saline (n=5)	41	48	47	45

Night cycle

Carbachol (n=5)	15	35	18	19
Scopolamine (n=5)	27	23	18	31
Saline (n=5)	21	32	23	29

Table 8

Mean length of W episodes (±SEM), in minutes, during the day and night

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=5)	2.3 (.10)	2.5 (.30)	3.0 (.44)	3.3 (.33)
Scopolamine (n=5)	3.4 (.44)	4.2 (.49)	4.2 (.42)	4.5 (.43)
Saline (n=5)	3.4 (.30)	3.6 (.43)	3.4 (.31)	3.2 (.15)

Night cycle

Carbachol (n=5)	6.2 (1.43)	4.4 (.51)	6.8 (.79)	5.3 (.30)
Scopolamine (n=5)	6.0 (.61)	8.7 (1.14)	9.1 (1.02)	8.5 (2.98)
Saline (n=5)	7.4 (.75)	5.9 (.65)	6.4 (.80)	6.3 (.92)

Table 9

Mean length of SWS episodes (±SEM), in minutes, during the day and night cycle.

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=5)	4.1 (.45)	3.3 (.28)	3.8 (.31)	4.3 (.48)
Scopolamine (n=5)	4.8 (.22)	6.5 (.46)	6.3 (.50)	4.7 (.36)
Saline (n=5)	4.7 (.11)	4.9 (.41)	5.0 (.35)	4.7 (.18)

Night cycle

Carbachol (n=5)	2.5 (.52)	2.5 (.32)	2.7 (.34)	3.0 (.19)
Scopolamine (n=5)	3.3 (.24)	4.7 (.36)	4.1 (.32)	3.9 (.57)
Saline (n=5)	3.6 (.25)	3.5 (.22)	3.4 (.22)	3.3 (.25)

Table 10

Mean length of PS episodes (±SEM), in minutes, during the day and night.

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=5)	1.9 (.08)	1.5 (.10)	1.6 (.12)	1.8 (.17)
Scopolamine (n=5)	2.0 (.09)	1.3 (.10)	1.5 (.08)	1.8 (.10)
Saline (n=5)	1.9 (.10)	1.8 (.06)	1.8 (.07)	1.8 (.10)

Night cycle

Carbachol (n=5)	1.4 (.08)	1.2 (.09)	1.2 (.16)	1.4 (.16)
Scopolamine (n=5)	1.6 (.16)	1.3 (.09)	1.5 (.11)	1.5 (.16)
Saline (n=5)	1.4 (.07)	1.3 (.09)	1.4 (.11)	1.5 (.08)

Table 11

Percent TST (± SEM) during the first 24 hours of pump operation for every group in the experiment. Also noted is the percent deviation from pooled baseline.

Group	Day	% change from pooled baseline	Night	% change from pooled baseline
Anterior to genu VII				
Carbachol (n=5)	45.9 (5.5)	-31%*	52.8 (4.6)	+52%*
Scopolamine (n=5)	40.0 (3.7)	-40%*	50.3 (4.7)	+15%
Saline (n=5)	45.3 (5.1)	-37%*	47.3 (6.7)	+17%
Caudal to genu VII				
Carbachol				
a) midline ponto-medullary (n=8)	35.7 (4.5)	-48%*	36.6 (6.3)	-3%
b) Left-side ponto-medullary (n=5)	53.1 (6.0)	-15%*	57.9 (5.1)	+35%*
Scopolamine (n=5)	47.8 (6.7)	-31%*	48.3 (6.6)	+36%*
Saline (n=6)	41.5 (7.6)	-41%*	41.9 (2.6)	+19%
Fourth Ventricle				
Carbachol (n=4)	42.5 (11.1)	-39%*	36.7 (9.3)	+19%
Scopolamine (n=4)	45.9 (7.1)	-30%*	48.1 (7.5)	+15%
Saline (n=4)	49.7 (3.8)	-17%	51.2 2.6)	+26%

* p=5%

Table 12

Percent SWS of total time (± SEM) during the first 24 hours of pump operation. Also noted is the percent deviation from within group pooled baseline.

Group	Day	% change from pooled baseline	Night	% change from pooled baseline
Anterior to genu VII				
Carbachol (n=5)	44.6 (5.3)	-22%*	46.3 (4.2)	+47%*
Scopolamine (n=5)	39.5 (3.8)	-31%*	46.6 (4.7)	+25%*
Saline (n=5)	43.6 (4.5)	-28%*	40.2 (4.7)	+14%
Caudal to genu VII				
Carbachol				
a) midline ponto-medullary (n=8)	35.2 (4.3)	-39%*	34.4 (5.9)	+4%
b) Left-side ponto-medullary (n=5)	50.3 (5.1)	-7%	47.8 (3.8)	+33%*
Scopolamine (n=5)	46.9 (6.5)	-20%*	45.6 (5.3)	+41%*
Saline (n=6)	40.2 (7.2)	-32%*	37.5 (2.4)	+18%
Fourth Ventricle				
Carbachol (n=4)	40.8 (10.3)	-29%*	32.8 (7.3)	-17%
Scopolamine (n=4)	44.6 (6.7)	-27%*	44.8 (6.6)	+20%
Saline (n=4)	47.3 (3.9)	-9%	45.9 (2.9)	+28%

* p=5%

Table 13

Percent PS of total time (± SEM) during the first 24 hours of pump operation. Also noted is the percent deviation from within group pooled baseline.

Group	Day	% change from pooled baseline	Night	% change from pooled baseline
Anterior to genu VII				
Carbachol (n=5)	1.3 (0.5)	-85%*	6.5 (1.2)	+103%*
Scopolamine (n=5)	0.4 (0.3)	-96%*	3.5 (1.1)	-44%*
Saline (n=5)	1.7 (0.8)	-85%*	7.1 (2.1)	+45%
Caudal to genu VII				
Carbachol				
a) midline ponto-medullary (n=8)	0.5 (0.2)	-95%*	2.2 (1.1)	-48%
b) Left-side ponto-medullary (n=5)	2.8 (1.5)	-67%*	10.1 (1.7)	+48%*
Scopolamine (n=5)	0.8 (0.4)	-93%*	2.7 (2.3)	-7%
Saline (n=6)	1.3 (0.5)	-88%*	4.3 (0.9)	+27%
Fourth Ventricle				
Carbachol (n=4)	1.7 (1.6)	-86%*	3.9 (2.6)	-34%
Scopolamine (n=4)	1.2 (0.8)	-89%*	3.3 (1.7)	-25%
Saline (n=4)	2.4 (0.7)	-71%*	5.4 (0.8)	+10%

* p=5%

TABLE 14

Summary of percent TST (±SEM) over 24 hours

24 hr cycle

	Baseline + Post-experimental	2-5	6-Post-drug 2
Carbachol			
a) midline ponto medullary (n=8)	53.2 (1.3)	51.5 (4.0)	51.8 (1.6)
b) Left-side ponto-medullary (n= 5)	52.5 (1.7)	52.2 (2.9)	49.9 (1.9)
Scopolamine (n= 5)	52.2 (1.0)	55.0 (2.4)	46.6 (3.5)
Saline (n= 6)	53.2 (2.1)	54.4 (2.4)	51.7 (2.0)

TABLE 15

Summary of SWS of total time (±SEM) over 24 hours

24 hr cycle

	Baseline + Post-experimental	2-5	6-Post-drug 2
Carbachol			
a)midline ponto medullary (n= 8)	45.7 (1.1)	43.0 (3.2)	43.3 (1.3)
b)Left-side ponto-medullary (n= 5)	44.9 (1.4)	44.1 (2.8)	42.5 (2.0)
Scopolamine (n= 5)	45.3 (1.0)	51.5 (2.3)	42.3 (3.7)
Saline (n= 6)	45.9 (2.0)	46.6 (2.0)	44.8 (1.6)

TABLE 16

Summary of percent TST (±SEM) during the day and night cycle

	Baseline + Post-experimental	2-5	6-Post drug 2
Day Cycle			
Carbachol			
a) midline ponto-medullary (n=8)	68.7 (2.9)	61.6 (7.6)	67.5 (4.0)
b) Left-side ponto-medullary (n= 5)	62.5 (3.2)	61.8 (5.0)	63.3 (3.1)
Scopolamine (n=5)	69.3 (1.7)	63.4 (3.2)	61.2 (3.3)
Saline (n= 6)	70.4 (2.4)	68.2 (2.6)	68.5 (3.4)
Night Cycle			
Carbachol			
a) midline ponto-medullary (n= 8)	37.8 (2.2)	42.5 (3.7)	37.3 (5.0)
b) Left-side ponto-medullary (n= 5)	42.8 (2.7)	42.8 (3.3)	36.3 (3.1)
Scopolamine (n= 5)	35.4 (2.1)	45.9 (3.3)	31.1 (4.5)
Saline (n=6)	35.2 (2.9)	40.6 (4.4)	34.1 (2.8)

TABLE 17

Summary of percent SWS of total time (±SEM) during the day and night

Day Cycle

	Baseline + Post-experimental	2-5	6-Post drug 2
Carbachol a)midline ponto-medullary (n= 8)	57.9 (2.3)	49.7 (5.9)	55.4 (2.9)
b) Left-side ponto-medullary (n= 5)	54.0 (2.6)	52.3 (4.5)	54.2 (2.9)
Scopolamine (n= 5)	58.4 (1.6)	59.7 (3.2)	55.4 (3.7)
Saline (n= 6)	59.5 (2.3)	57.4 (2.4)	58.1 (2.8)

Night Cycle

Carbachol a)midline ponto-medullary (n= 8)	33.1 (1.8)	36.3 (2.9)	32.2 (4.0)
b) Left-side ponto-medullary (n= 5)	36.0 (1.7)	36.8 (3.1)	31.6 (2.5)
Scopolamine (n=5)	32.4 (1.9)	43.5 (2.9)	28.3 (4.1)
Saline (n= 6)	31.8 (2.4)	36.6 (3.6)	31.8 (2.3)

Table 18

Average frequency of W episodes during the day and night cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Day cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	64	67	65	60
b) Left-side ponto-medullary (n=5)	68	89	90	93
Scopolamine (n=5)	61	63	57	60
Saline (n=6)	63	66	74	61
Night cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	66	68	62	62
b) Left-side ponto-medullary (n=5)	74	84	72	73
Scopolamine (n=5)	60	51	39	61
Saline (n=6)	65	64	64	58

Table 19

Average frequency of SWS episodes during the day and night cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Day cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	89	92	94	82
b) Left-side ponto-medullary (n=5)	88	105	112	105
Scopolamine (n=5)	88	72	71	83
Saline (n=6)	92	90	94	86
Night cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	71	80	67	67
b) Left-side ponto-medullary (n=5)	82	97	81	82
Scopolamine (n=5)	69	56	40	60
Saline (n=6)	68	71	68	64

Table 20

Average frequency of PS episodes during the day and night cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Day cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	42	49	50	43
b) Left-side ponto-medullary (n=5)	35	47	48	37
Scopolamine (n=5)	45	20	27	39
Saline (n=6)	46	46	43	44
Night cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	19	34	24	21
b) Left-side ponto-medullary (n=5)	40	43	34	31
Scopolamine (n=5)	20	17	14	12
Saline (n=6)	17	26	16	17

Table 21

Mean length of W episodes (±SEM), in minutes, during the day and night

	Baseline	2-5	6-Post drug 2	Post-experimental
Day cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	3.8 (.46)	4.3 (.63)	3.7 (.45)	4.0 (.42)
b) Left-side ponto-medullary (n=5)	4.2 (.53)	3.1 (.51)	3.2 (.48)	2.9 (.31)
Scopolamine (n=5)	3.3 (.10)	4.4 (.63)	6.2 (1.57)	4.0 (.30)
Saline (n=6)	3.1 (.08)	3.6 (.29)	3.2 (.40)	3.7 (.42)
Night cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	7.2 (.60)	6.4 (.68)	7.4 (.87)	7.2 (.25)
b) Left-side ponto-medullary (n=5)	5.4 (.66)	5.9 (1.37)	7.4 (1.62)	6.2 (.57)
Scopolamine (n=5)	7.6 (.72)	8.0 (.95)	15.0 (3.88)	8.4 (1.03)
Saline (n=6)	7.4 (.42)	7.0 (.87)	7.6 (.94)	8.3 (1.20)

Table 22

Mean length of SWS episodes (±SEM), in minutes, during the day and night cycle.

	Baseline	2-5	6-Post drug 2	Post-experimental
Day cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	4.7 (.33)	3.9 (.35)	4.3 (.23)	5.1 (.31)
b) Left-side ponto-medullary (n=5)	4.2 (.28)	3.5 (.29)	3.7 (.43)	3.8 (.41)
Scopolamine (n=5)	4.9 (.25)	6.0 (.44)	6.1 (.59)	4.9 (.23)
Saline (n=6)	4.8 (.38)	4.7 (.28)	4.5 (.22)	5.0 (.13)
Night cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	3.3 (.16)	3.3 (.24)	3.3 (.21)	3.7 (.12)
b) Left-side ponto-medullary (n=5)	3.5 (.32)	2.8 (.19)	2.8 (.27)	3.0 (.14)
Scopolamine (n=5)	3.8 (.31)	5.5 (.54)	5.5 (.94)	3.6 (.11)
Saline (n=6)	3.3 (.09)	3.7 (.31)	3.3 (.27)	3.9 (.23)

Table 23

Mean length of PS episodes (±SEM), in minutes, during the day and night.

	Baseline	2-5	6-Post drug 2	Post-experimental
Day cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	1.8 (.08)	1.6 (.12)	1.8 (.11)	1.9 (.12)
b) Left-side ponto-medullary (n=5)	1.8 (.14)	1.4 (.06)	1.4 (.09)	1.7 (.06)
Scopolamine (n=5)	1.8 (.08)	1.3 (.15)	1.5 (.20)	1.9 (.17)
Saline (n=6)	1.8 (.12)	1.7 (.13)	1.8 (.11)	1.8 (.10)
Night cycle				
Carbachol				
a) Midline ponto-medullary (n=8)	1.5 (.09)	1.3 (.13)	1.4 (.12)	1.5 (.14)
b) Left-side ponto-medullary (n=5)	1.4 (.08)	1.2 (.07)	1.3 (.11)	1.3 (.06)
Scopolamine (n=5)	1.4 (.08)	1.4 (.13)	1.4 (.21)	1.5 (.08)
Saline (n=6)	1.4 (.11)	1.4 (.11)	1.4 (.11)	1.6 (.14)

TABLE 24

Summary of percent TST (±SEM) over 24 hours

24 hr cycle

	Baseline + Post-experimental	2-5	6-Post-drug 2
Carbachol (n= 4)	57.3 (1.6)	55.3 (2.0)	52.3 (2.5)
Scopolamine (n=4)	53.5 (1.9)	55.0 (1.5)	54.1 (3.6)
Saline (n= 4)	55.8 (2.9)	55.7 (3.4)	61.2 (2.9)

TABLE 25

Summary of SWS of total time (±SEM) over 24 hours

24 hr cycle

	Baseline + Post-experimental	2-5	6-Post-drug 2
Carbachol (n=4)	48.3 (1.8)	46.0 (1.7)	44.0 (2.3)
Scopolamine (n=4)	46.1 (1.7)	49.9 (1.5)	48.8 (3.2)
Saline (n=4)	47.3 (2.0)	48.3 (3.0)	54.3 (3.2)

TABLE 26

Summary of percent TST (±SEM) over 24 hours

	Baseline + Post-experimental	2-5	6-Post-drug 2
Day Cycle			
Carbachol (n=4)	69.2 (2.7)	64.7 (4.3)	67.7 (3.2)
Scopolamine (n=4)	65.7 (3.1)	68.3 (2.4)	69.0 (3.5)
Saline (n=4)	59.9 (3.2)	65.2 (4.0)	69.3 (3.2)
Night Cycle			
Carbachol (n=4)	45.3 (1.3)	46.0 (2.7)	35.5 (3.4)
Scopolamine (n=4)	41.8 (2.7)	41.6 (2.3)	37.2 (3.0)
Saline (n=4)	40.7** (5.2)	46.1 (3.9)	52.9 (4.7)

**from baseline

TABLE 27

Summary of percent SWS of total time (±SEM) during the day and night

	Baseline + Post-experimental	2-5	6-Post-drug 2
Day Cycle			
Carbachol (n= 4)	57.2 (2.8)	52.3 (3.4)	55.6 (2.6)
Scopolamine (n= 4)	61.1 (2.0)	61.9 (2.1)	62.3 (3.1)
Saline (n= 4)	51.7 (2.0)	55.9 (3.5)	60.8 (2.9)
Night Cycle			
Carbachol (n= 4)	39.3 (1.3)	39.7 (2.1)	31.5 (2.7)
Scopolamine (n= 4)	37.4 (2.2)	37.7 (2.1)	33.3 (2.5)
Saline (n=4)	35.8** (4.7)	40.7 (3.2)	47.7 (4.7)

**baseline only

Table 28

Average frequency of W episodes during the day and night cycle

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=4)	66	73	73	61
Scopolamine (n=4)	76	69	56	72
Saline (n=4)	84	69	72	70

Night cycle

Carbachol (n=4)	90	72	75	83
Scopolamine (n=4)	83	51	44	74
Saline (n=4)	65	72	83	68

Table 29

Average frequency of SWS episodes during the day and night cycle

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=4)	103	102	99	83
Scopolamine (n=4)	94	79	70	83
Saline (n=4)	94	84	93	84

Night cycle

Carbachol (n=4)	99	85	78	92
Scopolamine (n=4)	87	57	46	79
Saline (n=4)	68	80	93	95

Table 30

Average frequency of PS episodes during the day and night cycle

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n= 4)	55	57	51	39
Scopolamine (n= 4)	40	32	29	34
Saline (n-4)	39	38	36	27

Night cycle

Carbachol (n= 4)	19	36	23	32
Scopolamine (n= 4)	21	22	17	24
Saline (n= 4)	23	28	26	52

Table 31

Mean length of W episodes (±SEM), in minutes, during the day and night

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n= 4)	3.2 (.49)	3.6 (.68)	3.2 (.46)	4.2 (.64)
Scopolamine (n= 4)	3.2 (.30)	3.4 (.33)	3.6 (.45)	3.4 (.43)
Saline (n= 4)	3.4 (.45)	3.7 (.51)	3.2 (.48	4.7 (.98)

Night cycle

Carbachol (n= 4)	4.6 (.45)	5.6 (.44)	6.2 (.77)	4.8 (.45)
Scopolamine (n= 4)	5.5 (.78)	8.3 (.66)	7.9 (1.97)	5.9 (1.14)
Saline (n= 4)	6.6 (.78)	5.5 (.62)	4.4 (.83)	4.1 (.66)

Table 32

Mean length of SWS episodes (±SEM), in minutes, during the day and night cycle.

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=4)	4.2 (.19)	3.7 (.18)	4.0 (.23)	5.0 (.56)
Scopolamine (n=4)	4.2 (.23)	5.7 (.50)	5.9 (.58)	4.5 (.39)
Saline (n=4)	4.3 (.42)	4.8 (.22)	4.9 (.45)	4.4 (.48)

Night cycle

Carbachol (n=4)	2.9 (.16)	3.5 (.35)	2.9 (.18)	3.1 (.20)
Scopolamine (n=4)	3.0 (.26)	4.8 (.32)	3.9 (.85)	3.7 (.32)
Saline (n=4)	3.8 (.34)	3.7 (.27)	3.8 (.43)	3.8 (.29)

Table 33

Mean length of PS episodes (±SEM), in minutes, during the day and night.

Day cycle

	Baseline	2-5	6-Post drug 2	Post-experimental
Carbachol (n=4)	1.8 (.07)	1.6 (.11)	1.7 (.06)	2.0 (.07)
Scopolamine (n=4)	2.0 (.16)	1.5 (.12)	1.5 (.12)	1.9 (.10)
Saline (n=4)	1.8 (.08)	1.8 (.17)	1.7 (.17)	1.9 (.11)

Night cycle

Carbachol (n=4)	1.8 (.17)	1.3 (.13)	1.4 (.18)	1.6 (.11)
Scopolamine (n=4)	1.4 (.08)	1.3 (.10)	1.2 (.25)	1.4 (.18)
Saline (n=4)	1.5 (.11)	1.4 (.05)	1.4 (.11)	1.7 (.10)

Table 34

Summary of percent TST, percent SWS and percent PS over 24 hours. The numbers in parentheses refer to the standard error of the mean.

Dependent variable	Baseline	2	4-10	12-Post drug 2	Post experiment
Percent TST	53.4 (2.61)	53.7 (0.81)	54.1 (2.03)	48.9 (2.29)	52.8 (3.96)
Percent SWS	45.7 (2.08)	45.8 (0.87)	45.2 (1.65)	41.3 (1.90)	44.9 (3.18)
Percent PS	7.7 (0.75)	7.9 (1.52)	8.9 (0.86)	7.6 (0.77)	7.9 (0.99)

Table 35

Percent TST and percent SWS of total time during the day and night cycles.

Day cycle

Dependent variable	Baseline	2	4-10	12-Post drug 2	Post-experiment
Percent TST	66.2 (2.59)	60.9 (3.35)	72.3 (3.18)	67.5 (2.98)	59.3 (5.13)
Percent SWS	56.6 (2.22)	50.1 (1.69)	58.6 (2.20)	54.7 (2.11)	51.4 (4.15)

Night cycle

Percent TST	40.9 (3.95)	46.6 (2.31)	37.4 (3.32)	34.7 (3.26)	45.9 (6.02)
Percent SWS	35.2 (3.26)	41.5 (1.90)	32.9 (2.40)	31.0 (2.45)	38.2 (4.25)

Table 36

Average frequency of wake, SWS and PS episodes during the day and night cycles.

Day cycle

Dependent variable	Baseline	2	4-10	12-Post drug 2	Post-experiment
Frequency of wake episodes	64	80	61	50	45
Frequency of SWS episodes	84	102	97	79	60
Frequency of PS episodes	40	54	58	46	28

Night cycle

Frequency of wake episodes	57	74	67	69	47
Frequency of SWS episodes	64	87	75	73	63
Frequency of PS episodes	27	35	26	22	31

Table 37

Mean length in minutes of wake, SWS and PS episodes during the day and night cycles. The numbers in parentheses refer to the standard error of the mean.

Day cycle

Dependent variable	Baseline	2	4-10	12-Post drug 2	Post-experiment
Mean length of wake episodes	3.8 (.50)	3.5 (.21)	3.1 (.36)	4.0 (.70	6.8 (1.52)
Mean length of SWS episodes	4.9 (.41)	3.6 .32)	4.1 (.21)	4.0 (.23)	6.1 (.60)
Mean length of PS episodes	1.8 (1.8)	1.4 (.08)	1.6 (.08)	1.6 (.09)	2.0 (.18)

Night cycle

Mean length of wake episodes	7.8 (1.21)	5.4 (.60	6.9 (.68)	7.0 (.85)	8.0 (1.65)
Mean length of SWS episodes	4.0 (.21)	3.5 (.25)	3.2 (.20)	3.1 (.19)	4.0 (.30)
Mean length of PS episodes	1.5 (.15)	1.1 (.06)	1.3 (.38)	1.2 (.08)	1.6 (.15)

Table 38

Summary of train-test percent avoidance scores for all 50 trials. Numbers in parentheses refer to the SEM

Retention testing at 7 days

	Train	Test
Carbachol (n=8)	42.0 (10.6)	67.5 * (7.8)
Scopolamine (n=8)	63.8 (8.4)	78.3 * (4.9)
Saline (n=8)	52.8 (12.6)	70.8 * (13.0)

Retention testing at 21 days

Carbachol (n=8)	39.((9.9	67.3 * (6.7)
Scopolamine (n=8)	50.3 (9.3)	83.5 * (4.3)
Saline (n=8)	37.3 (6.7)	73.0 * (6.6)

* p=.05 from training

Figure 1. Schematic representation of cannula placement at the level of the genu of the seventh nerve. The cannula is a chronically implanted 21 gauge stainless-steel tube and it is placed down the midline so as to insure maximal excitation of reticular neurons on either side. The coronal sections are adapted from Palkovits and Jacobowitz (1974). Note that carbachol increased PS at this level.

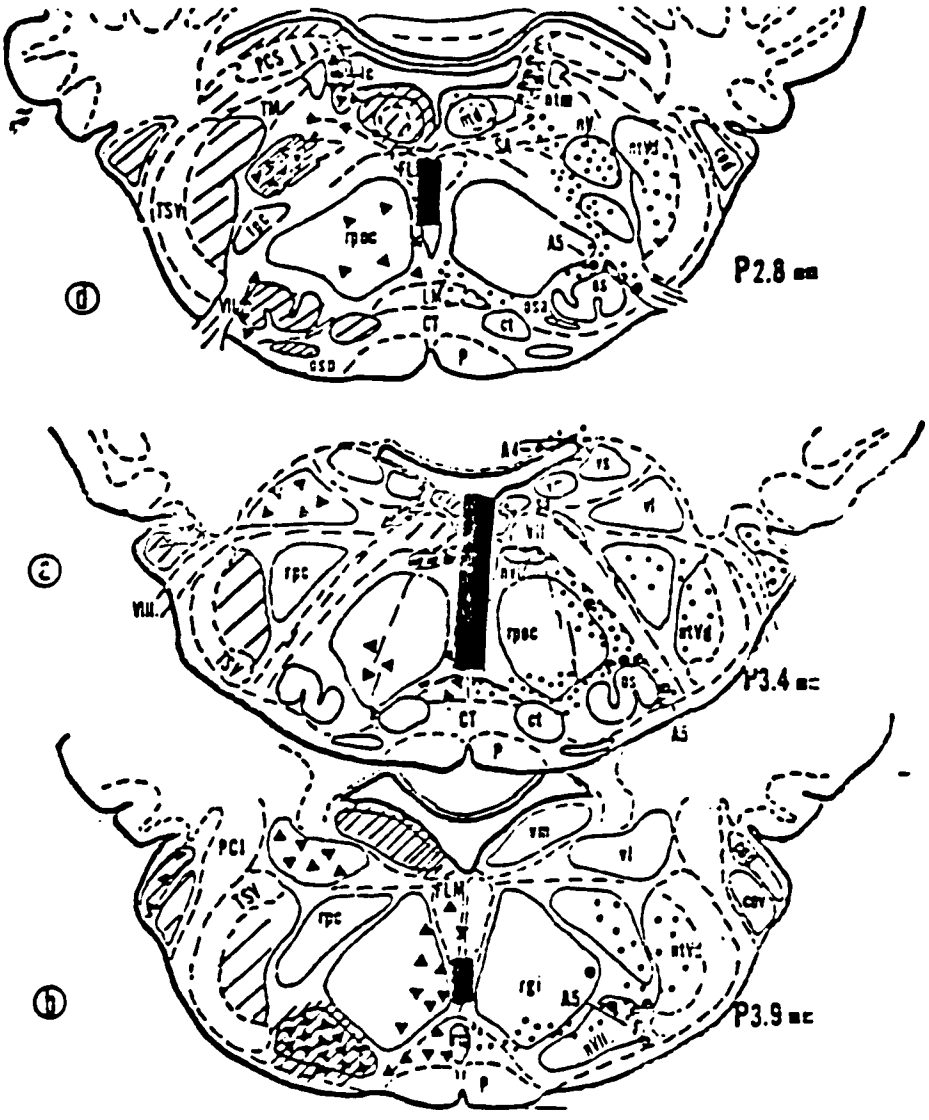

d
P2.8 mm
c
P3.4 mm
b
P3.9 mm

Figure 2. Schematic representation of cannula placement in areas caudal to the genu of the seventh nerve.

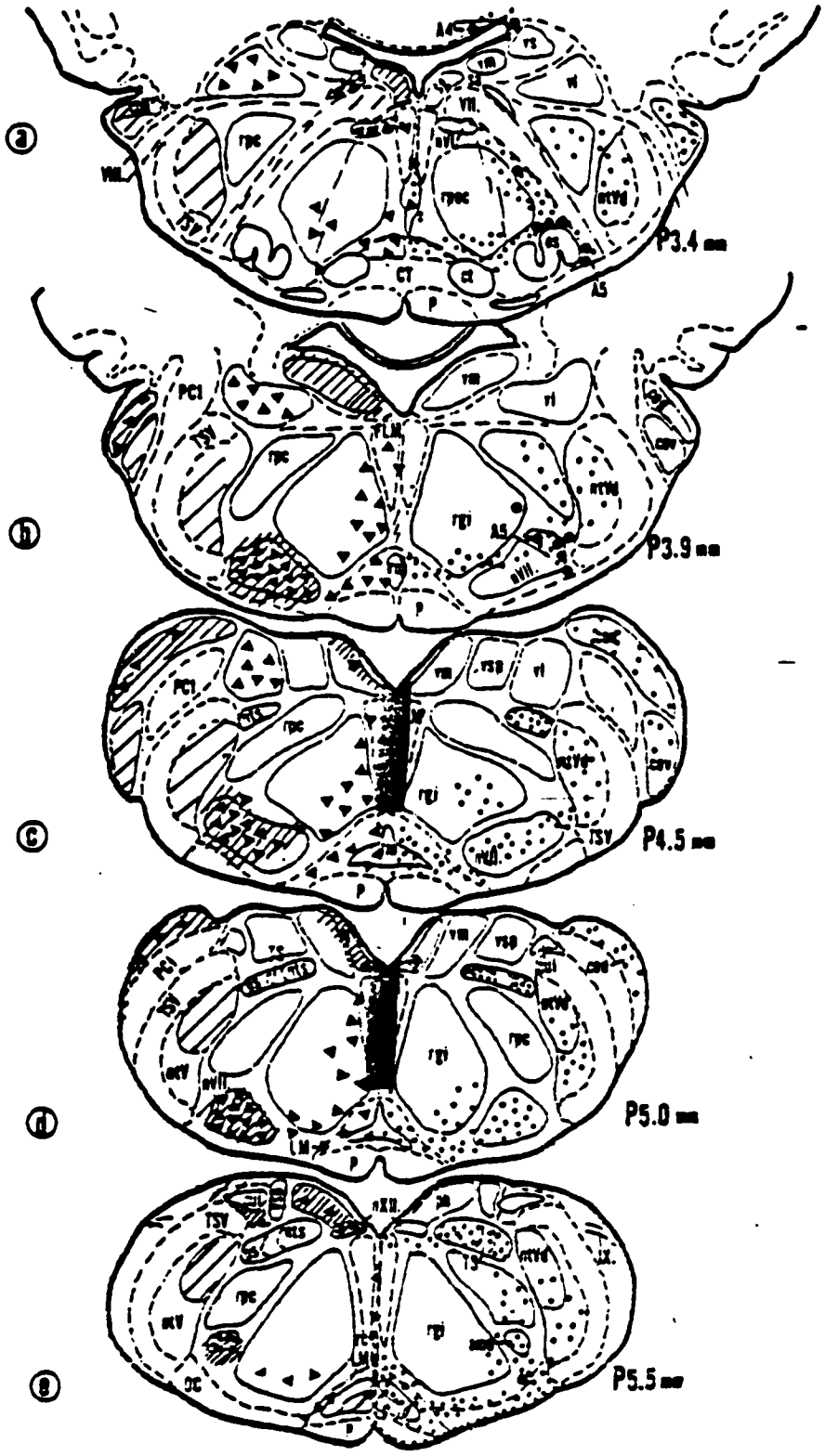

a
P3.4 mm
b
P3.9 mm
c
P4.5 mm
d
P5.0 mm
e
P5.5 mm

Figure 3. PS change as a result of carbachol, scopolamine or saline infusion. Independent groups of rats are administered the drugs or saline and 24 hour records obtained. Since this is a repeated design all comparisions are to the subjects own baseline. In each drug and saline condition, a 24 hr mean percent PS score is determined by pooling within subjects (i) baseline + post-experimental, (ii)days 2 through 5, and (iii) days 6 through post-drug 2. Each bar represents mean percent PS deviation from within subjects pooled baseline (ie. baseline + post-experimental).

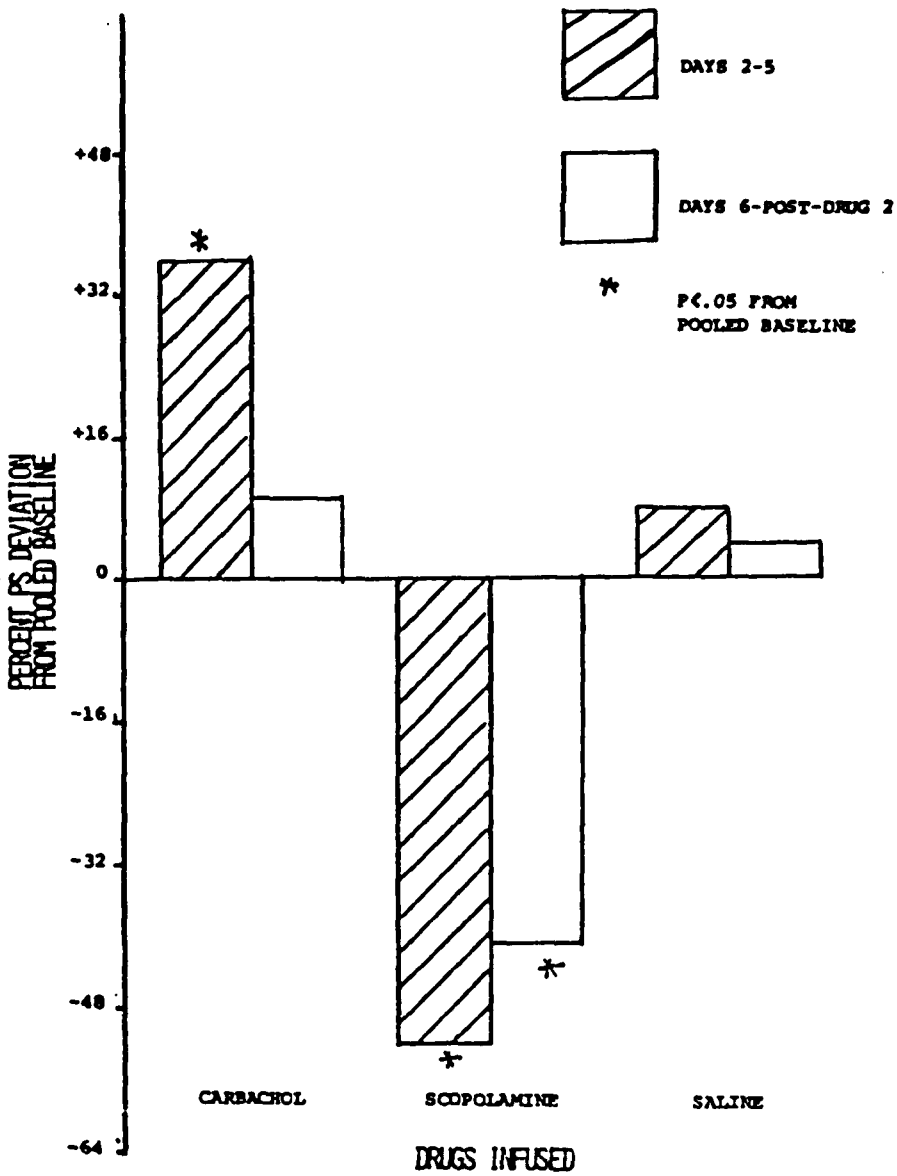

DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM POOLED BASELINE
+48
+32
+16
0
-16
-32
-48
-64
PERCENT PS DEVIATION FROM POOLED BASELINE
CARBACHOL
SCOPOLAMINE
SALINE
DRUGS INFUSED

Figure 4. 24 hr PS rhythmicity profiles as a function of chronic carbachol infusion. Each point represents the mean percent PS over 3 hrs. During the night cycle carbachol significantly increases PS.

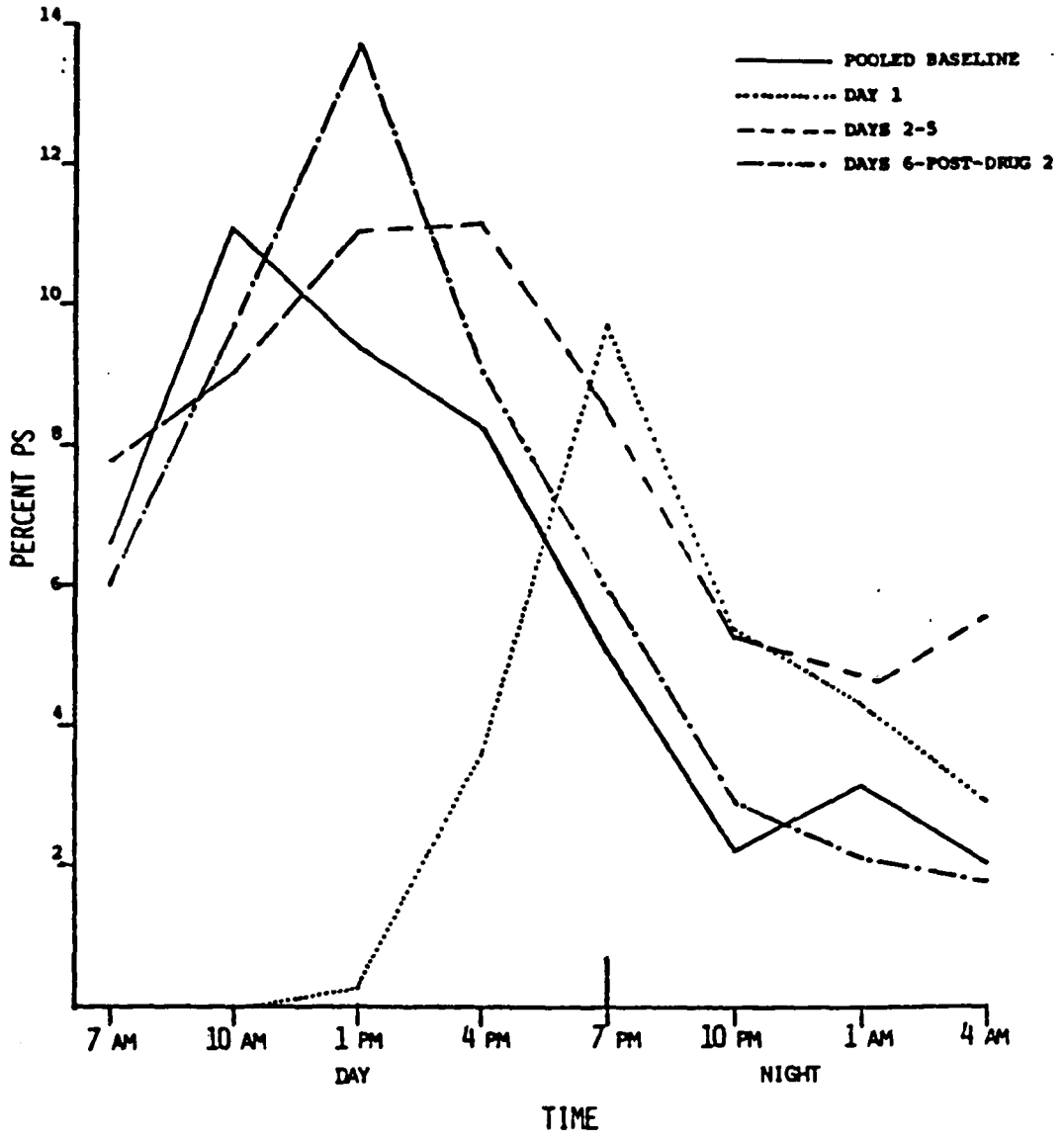

POOLED BASELINE
DAY 1
DAYS 2-5
DAYS 6-POST-DRUG 2
PERCENT PS
14
12
10
8
6
4
2
7 AM
10 AM
1 PM
4 PM
7 PM
10 PM
1 AM
4 AM
DAY
NIGHT
TIME

Figure 5. 24 hr PS rhythmicity profiles as a result of scopolamine infusion. The PS decrease occurs during the day cycle and there is no PS rebound.

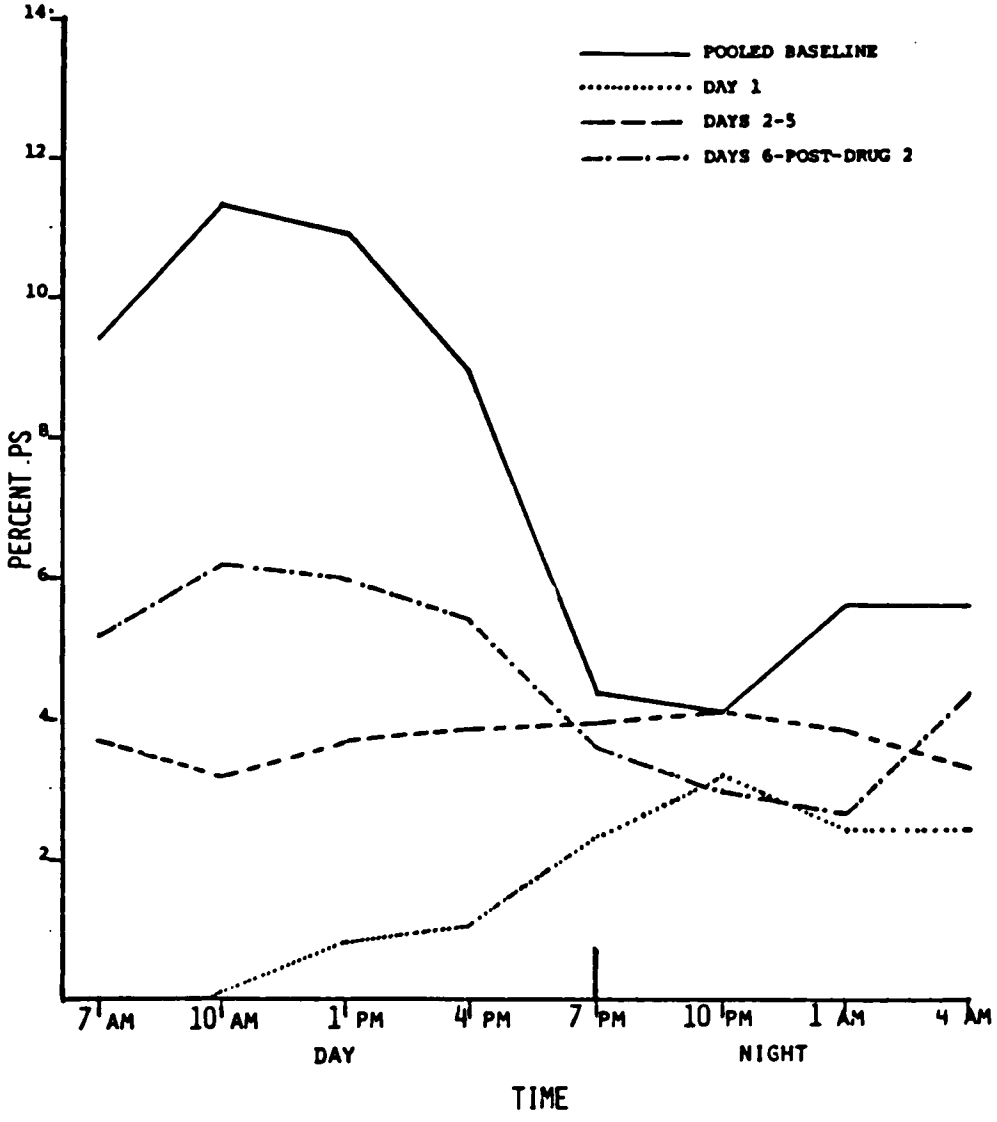

POOLED BASELINE
DAY 1
DAYS 2-5
DAYS 6-POST-DRUG 2
PERCENT PS
14
12
10
8
6
4
2
7 AM
10 AM
1 PM
4 PM
7 PM
10 PM
1 AM
4 AM
DAY
NIGHT
TIME

Figure 6. 24 hr PS rhythmicity profiles as a result of saline infusion.

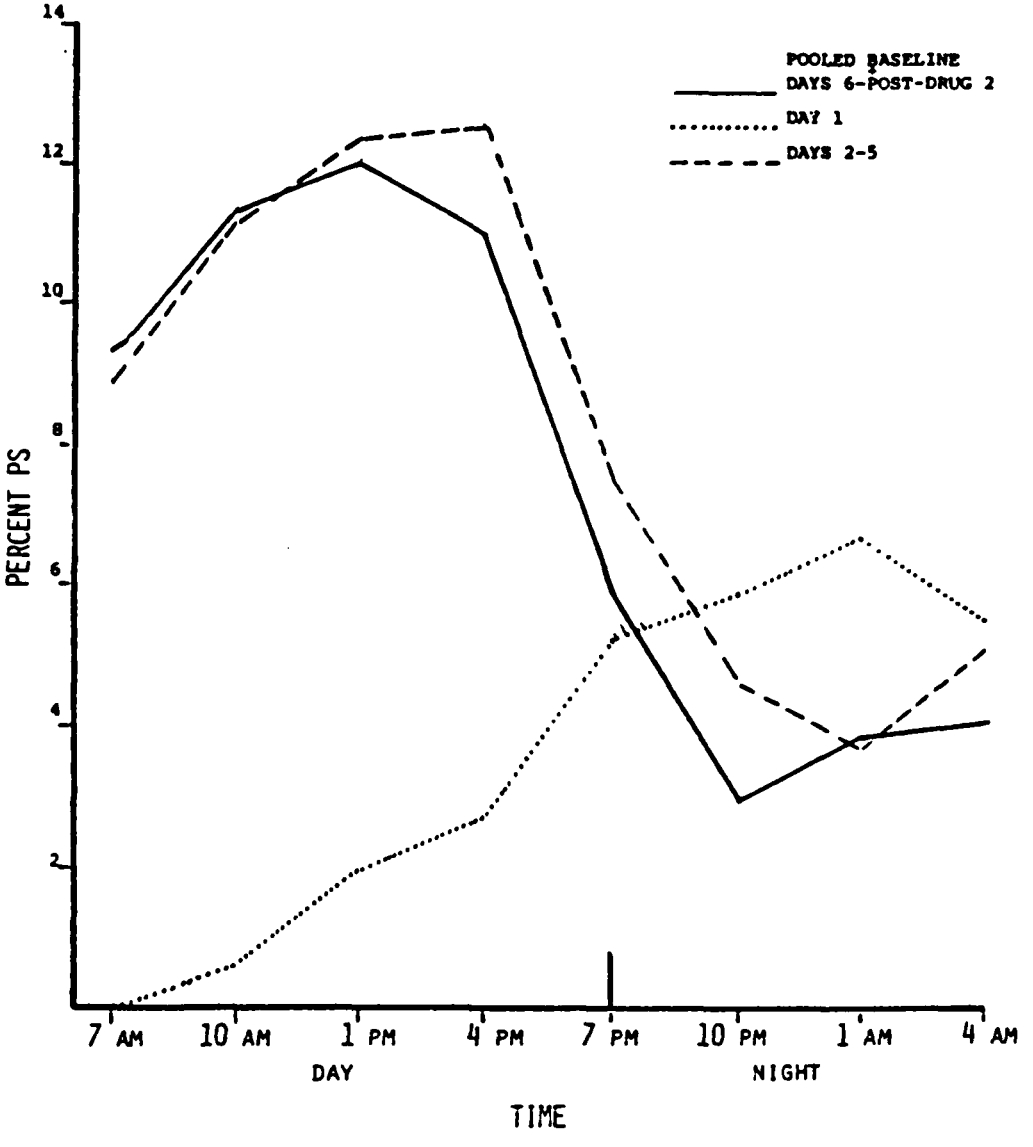

POOLED BASELINE
DAYS 6-POST-DRUG 2
DAY 1
DAYS 2-5
PERCENT PS
14
12
10
8
6
4
2
7 AM
10 AM
1 PM
4 PM
7 PM
10 PM
1 AM
4 AM
DAY
NIGHT
TIME

Figure 7. Day cycle percent change in PS as a result of carbachol, scopolamine or saline infusion. For additional details see Fig. 3 or text.

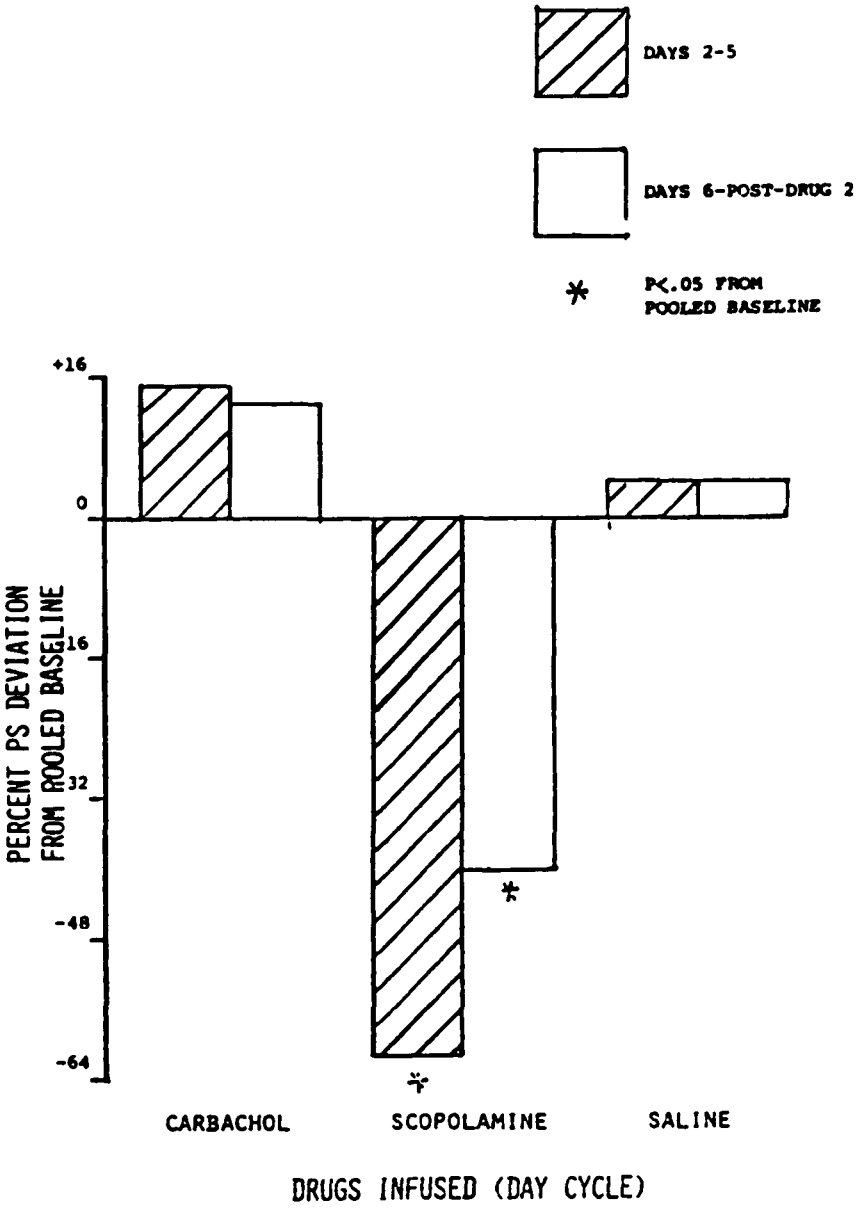

DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM POOLED BASELINE
+16
0
-16
-32
-48
-64
PERCENT PS DEVIATION FROM POOLED BASELINE
CARBACHOL
SCOPOLAMINE
SALINE
DRUGS INFUSED (DAY CYCLE)

Figure 8. Summary of PS decrease induced by scopolamine during each day cycle of infusion. Note the step-ladder restoration of PS to baseline levels which might indicate attempts by the receptor to achieve homeostasis.

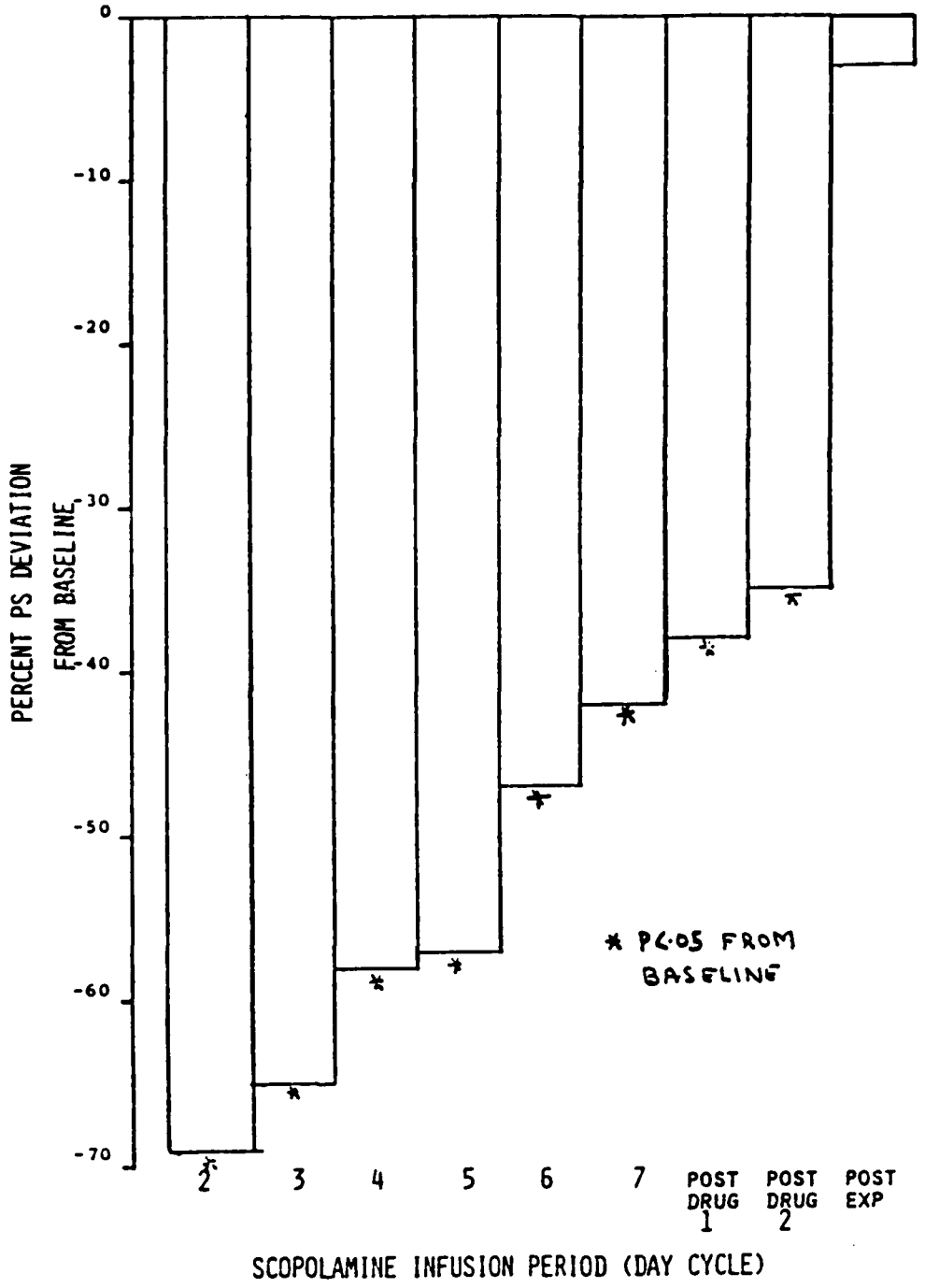

0
-10
-20
-30
-40
-50
-60
-70
PERCENT PS DEVIATION FROM BASELINE
2
3
4
5
6
7
POST DRUG 1
POST DRUG 2
POST EXP
* P<.05 FROM BASELINE
SCOPOLAMINE INFUSION PERIOD (DAY CYCLE)

Figure 9. Night cycle percent change in PS as a result of carbacnol, scopolamine or saline infusion. For additional details see Fig. 3 or text.

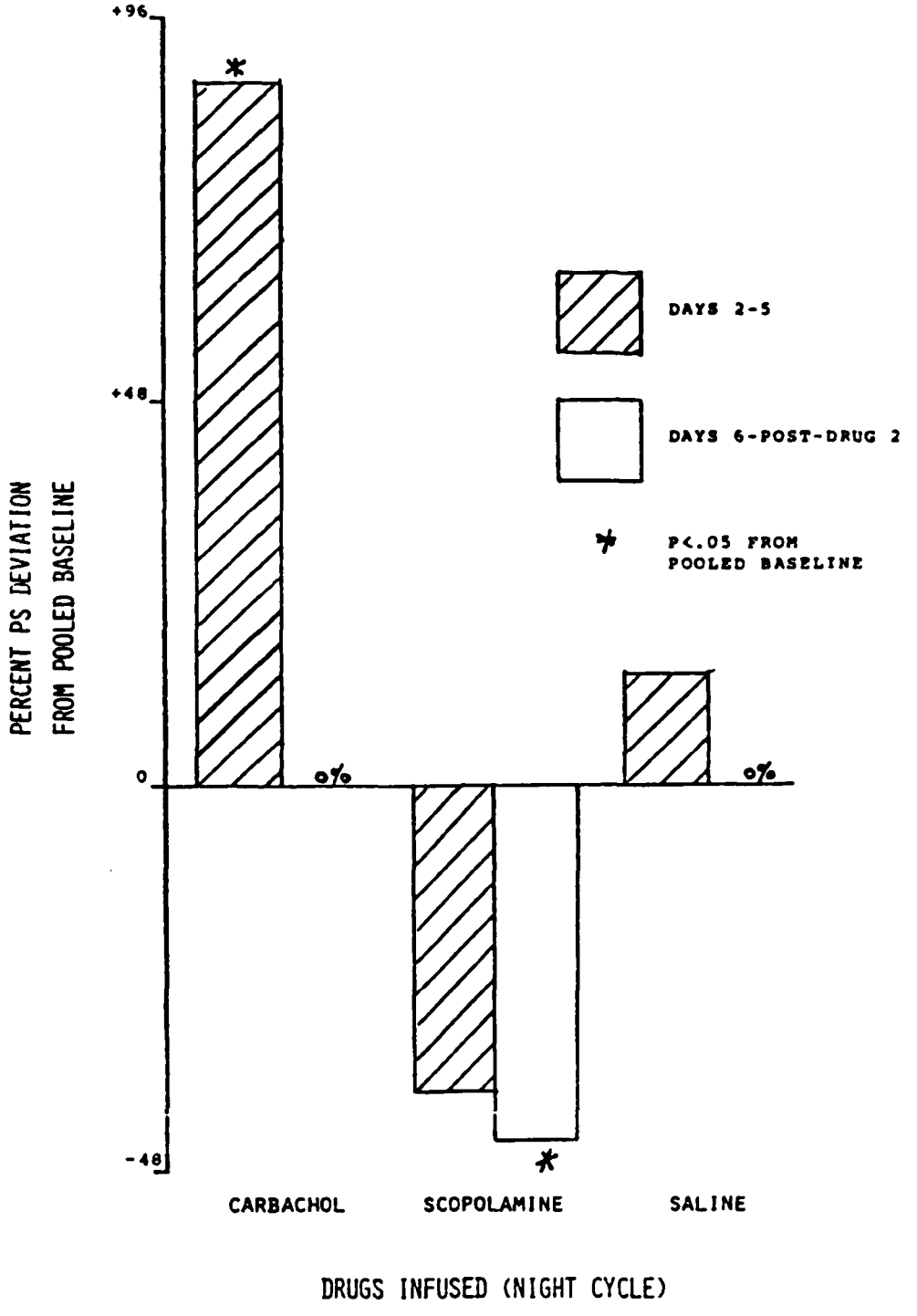

+96
+48
0
-48
PERCENT PS DEVIATION
FROM POOLED BASELINE
DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM
POOLED BASELINE
0%
0%
CARBACHOL
SCOPOLAMINE
SALINE
DRUGS INFUSED (NIGHT CYCLE)

Figure 10. Summary of PS increase produced by carbachol during each night cycle of drug infusion. After an initial increase, PS returns to baseline levels fairly rapidly.

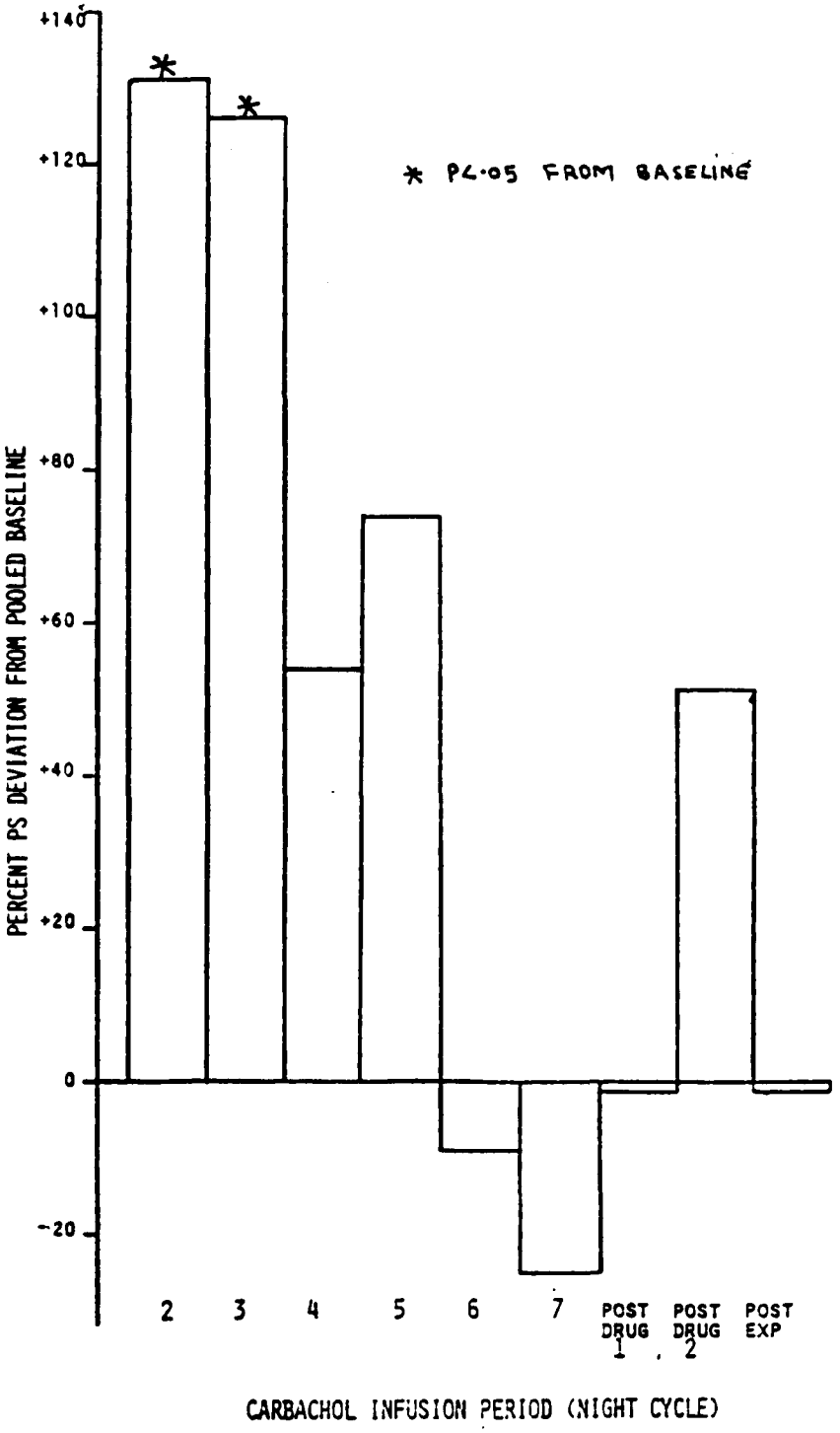

+140
+120
+100
+80
+60
+40
+20
0
-20
* P<.05 FROM BASELINE
PERCENT PS DEVIATION FROM POOLED BASELINE
2
3
4
5
6
7
POST DRUG 1
POST DRUG 2
POST EXP
CARBACHOL INFUSION PERIOD (NIGHT CYCLE)

Figure 11. Percent change in PS over 24 hrs as a result of drug or saline infusion.

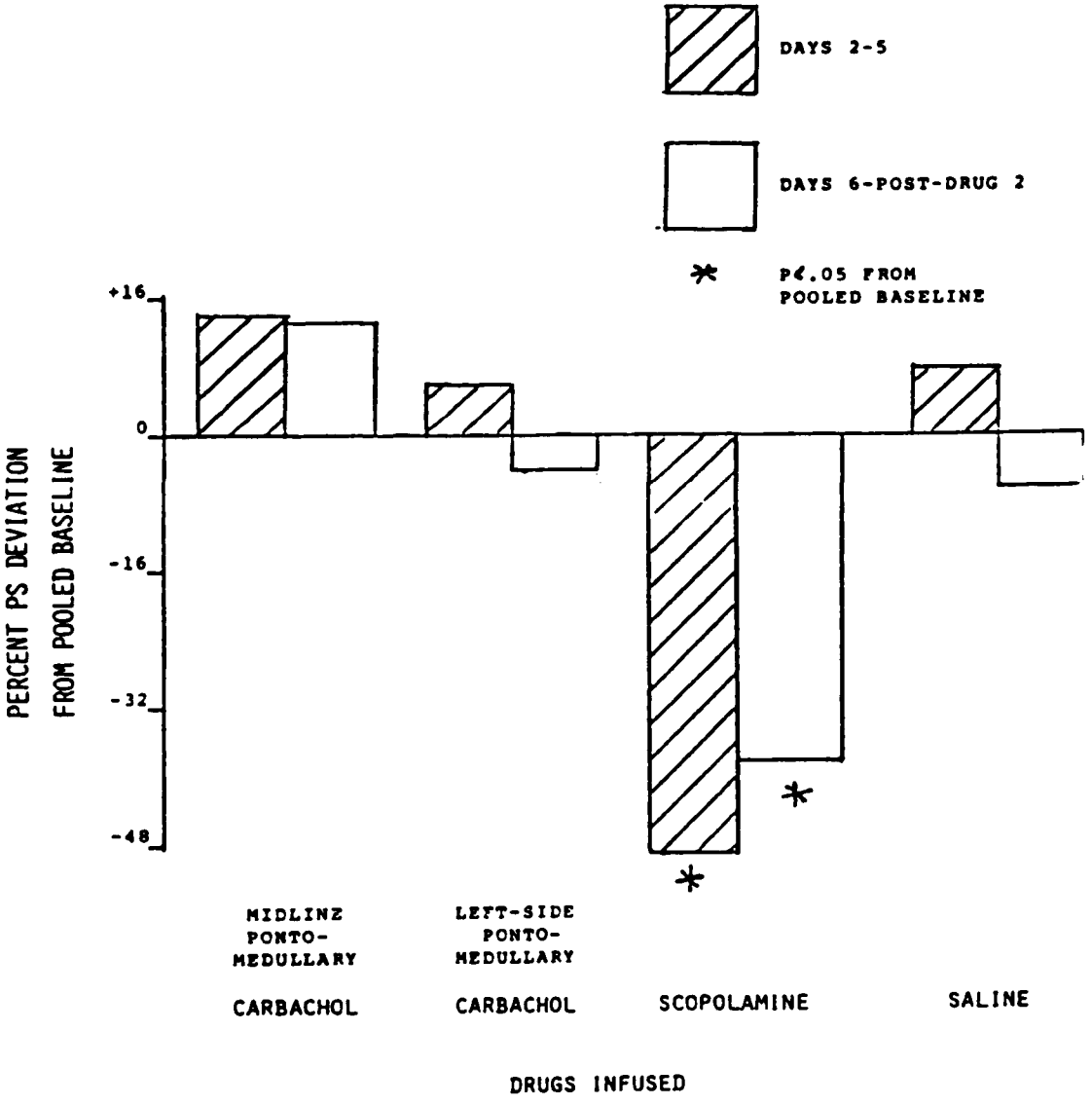

DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM POOLED BASELINE
+16
0
-16
-32
-48
PERCENT PS DEVIATION FROM POOLED BASELINE
MIDLINE PONTO-MEDULLARY CARBACHOL
LEFT-SIDE PONTO-MEDULLARY CARBACHOL
SCOPOLAMINE
SALINE
DRUGS INFUSED

Figure 12. Percent change in PS during the day cycle as a result of drug or saline infusion.

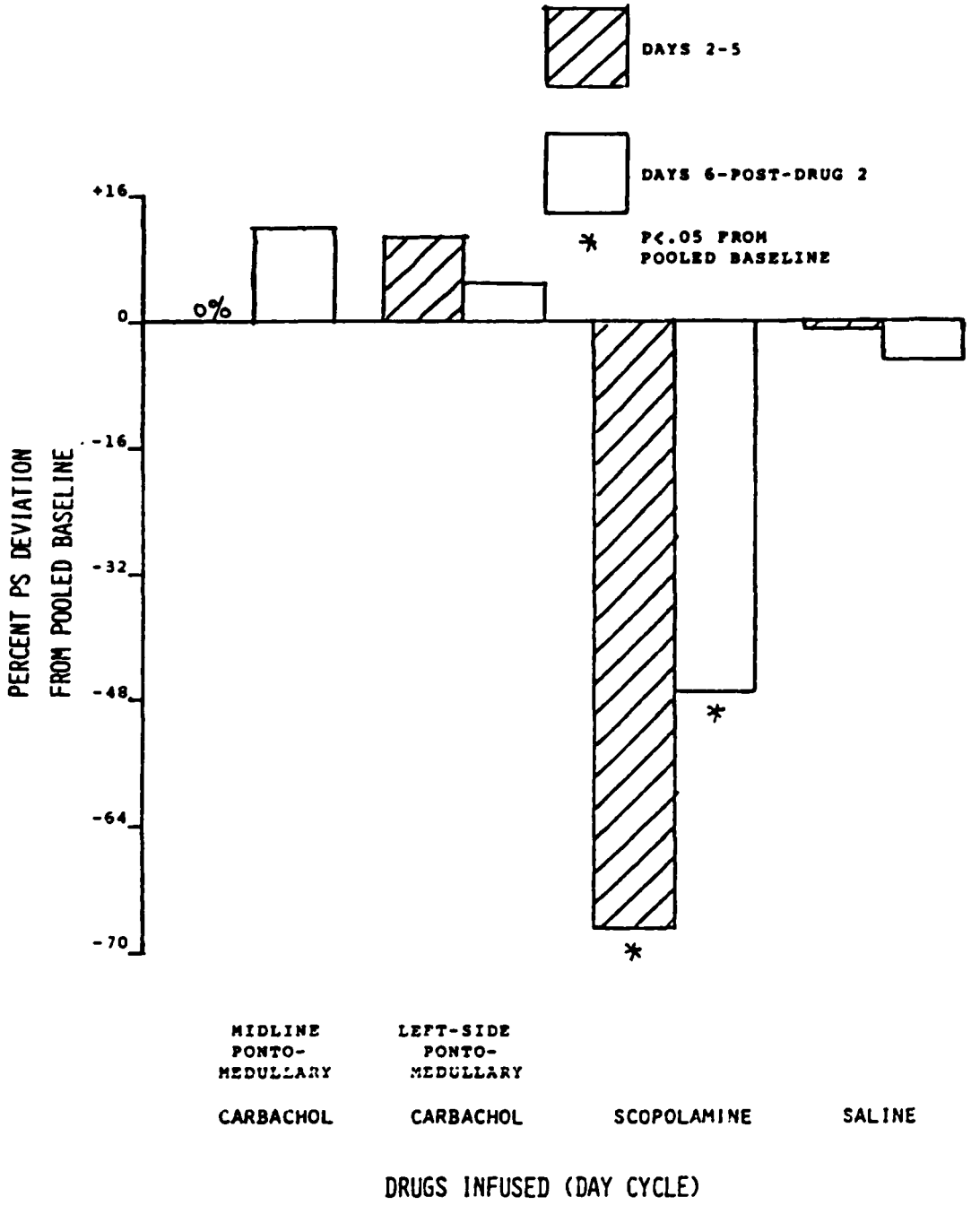

DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM POOLED BASELINE
+16
0
-16
-32
-48
-64
-70
0%
PERCENT PS DEVIATION FROM POOLED BASELINE
MIDLINE PONTO-MEDULLARY
LEFT-SIDE PONTO-MEDULLARY
CARBACHOL
CARBACHOL
SCOPOLAMINE
SALINE
DRUGS INFUSED (DAY CYCLE)

Figure 13. Summary of PS decrease produced by scopolamine during each night cycle of infusion. The gradual normalization of PS levels is similar to that in Fig. 8.

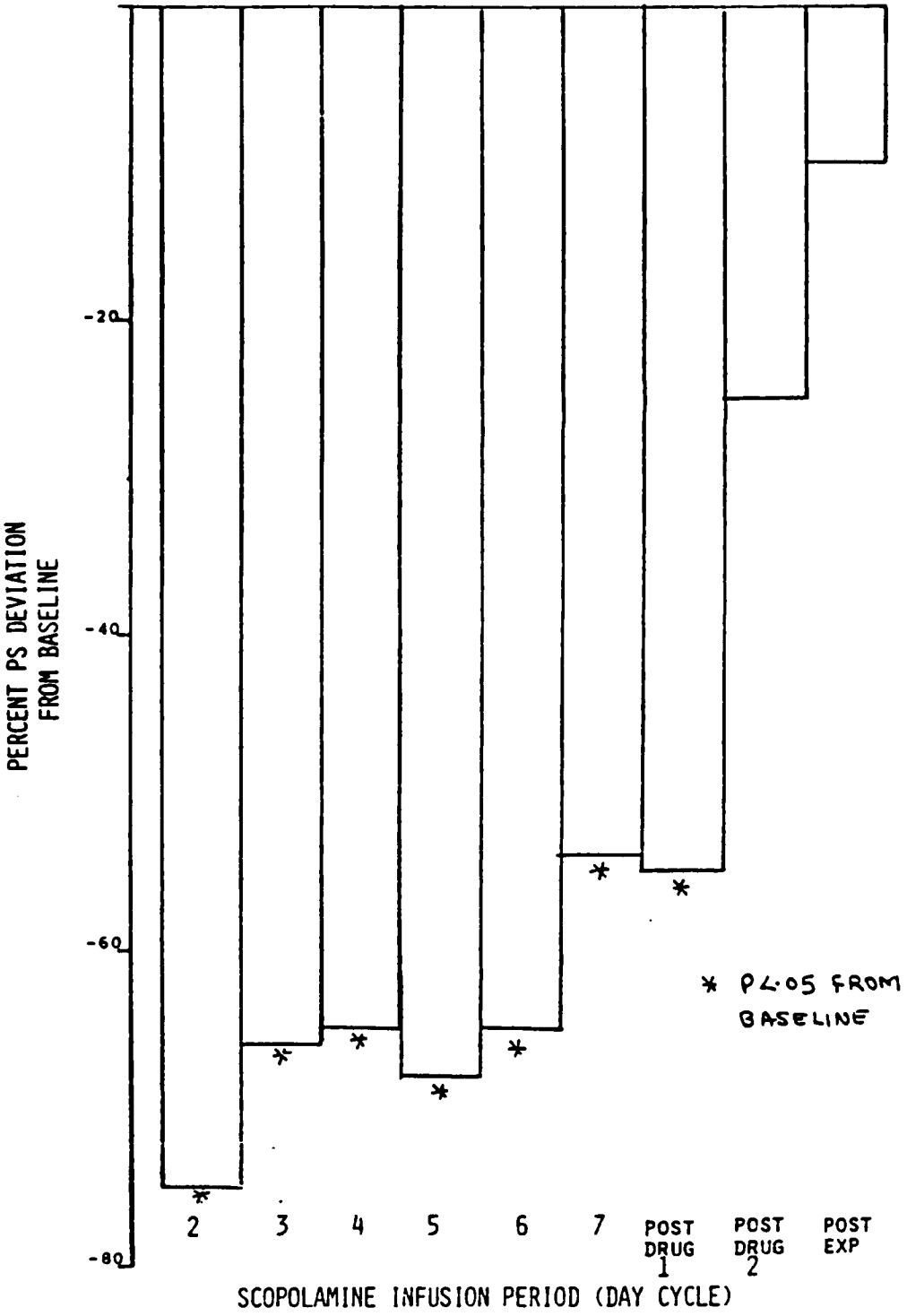

PERCENT PS DEVIATION FROM BASELINE
-20
-40
-60
-80
2
3
4
5
6
7
POST DRUG 1
POST DRUG 2
POST EXP
SCOPOLAMINE INFUSION PERIOD (DAY CYCLE)
* P<.05 FROM BASELINE

Figure 14. Percent change in PS during the night cycle as a result of drug or saline infusion.

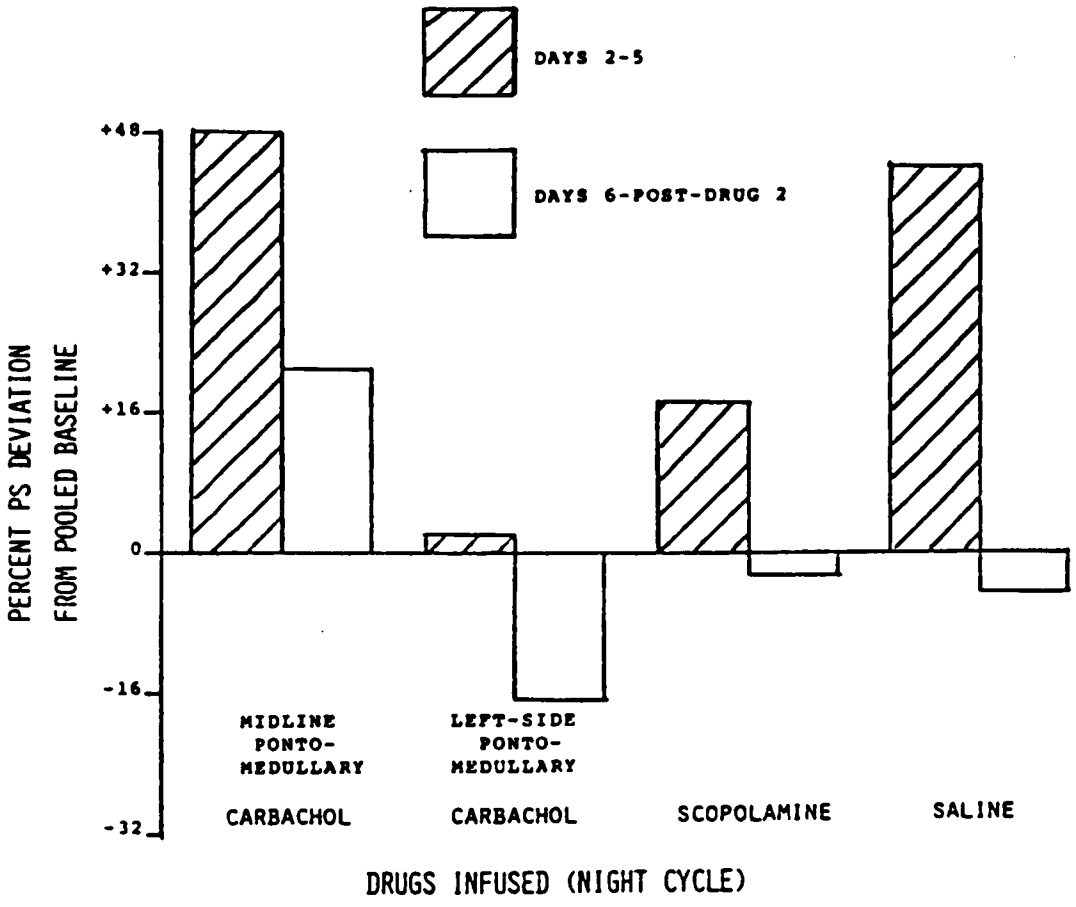

DAYS 2-5
DAYS 6-POST-DRUG 2
+48
+32
+16
0
-16
-32
PERCENT PS DEVIATION FROM POOLED BASELINE
MIDLINE PONTO-MEDULLARY
LEFT-SIDE PONTO-MEDULLARY
CARBACHOL
CARBACHOL
SCOPOLAMINE
SALINE
DRUGS INFUSED (NIGHT CYCLE)

Figure 15. PS change over 24 hrs as a result of drug or saline infusions into the fourth ventricle.

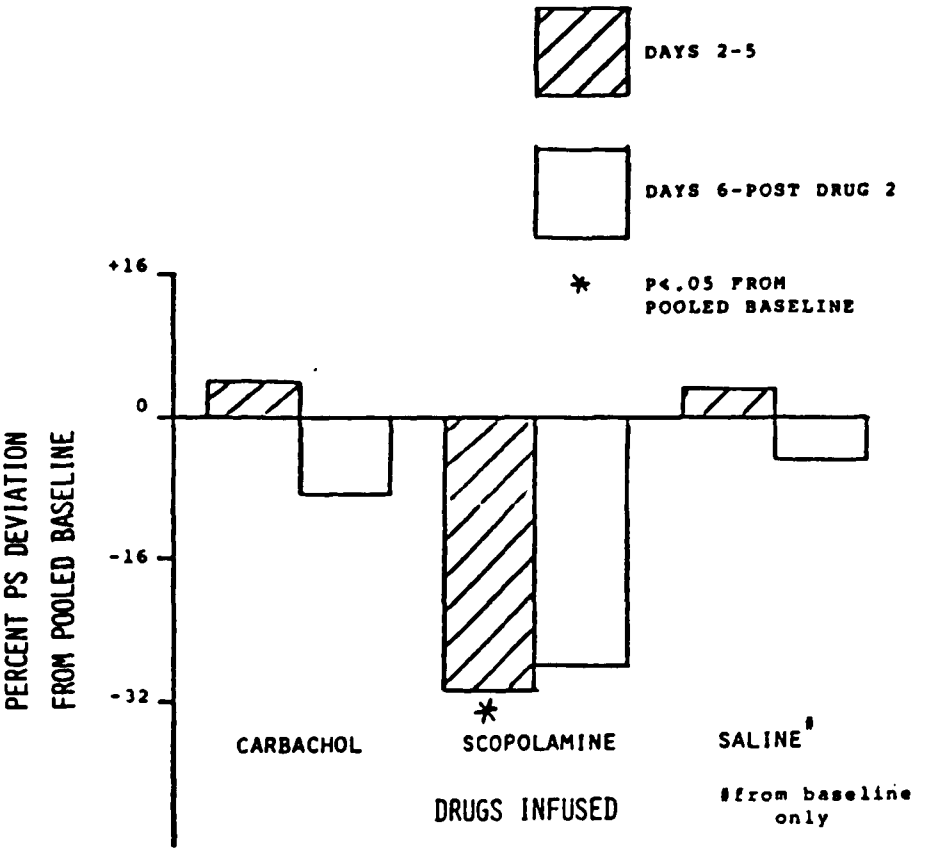

DAYS 2-5
DAYS 6-POST DRUG 2
* P<.05 FROM POOLED BASELINE
+16
0
-16
-32
PERCENT PS DEVIATION FROM POOLED BASELINE
CARBACHOL
SCOPOLAMINE
SALINE#
DRUGS INFUSED
#from baseline only

Figure 16. PS change during the day cycle as a result of drug or saline infusions into the fourth ventricle.

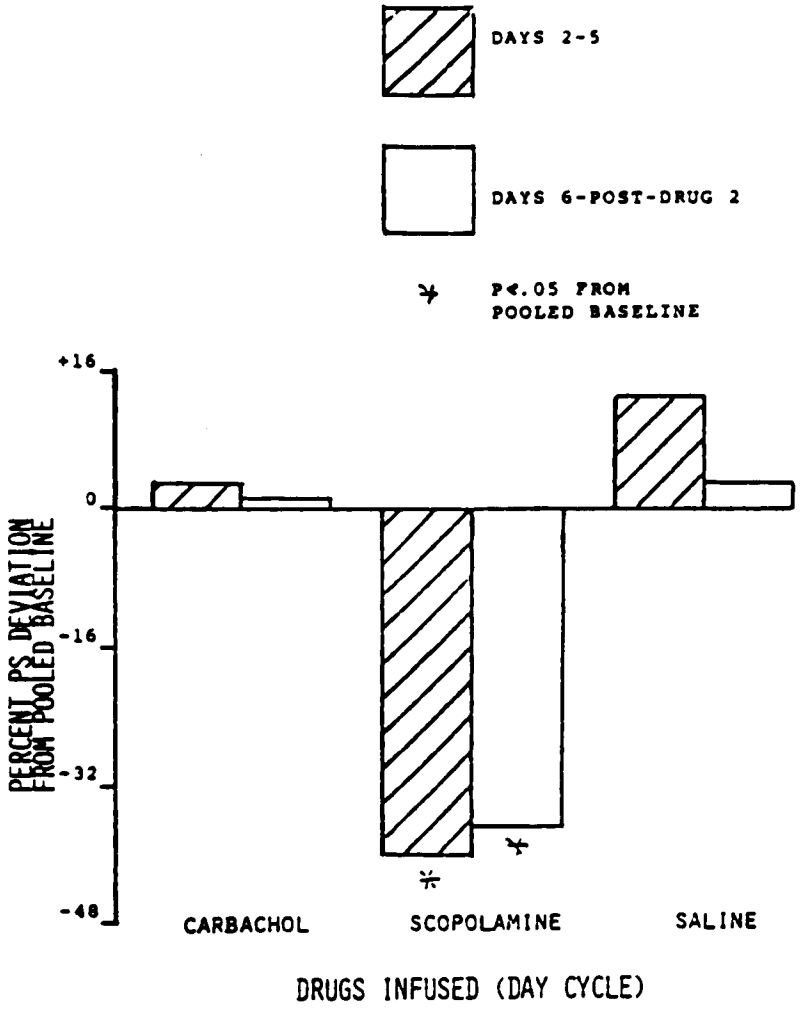

DAYS 2-5
DAYS 6-POST-DRUG 2
P<.05 FROM POOLED BASELINE
+16
0
-16
-32
-48
PERCENT PS DEVIATION FROM POOLED BASELINE
CARBACHOL
SCOPOLAMINE
SALINE
DRUGS INFUSED (DAY CYCLE)

Figure 17. Summary of PS decrease during each day cycle of scopolamine infusions into the fourth ventricle. The PS decrease is similar to that seen in the pons (Fig. 8), and the ponto-medullary region (Fig. 13).

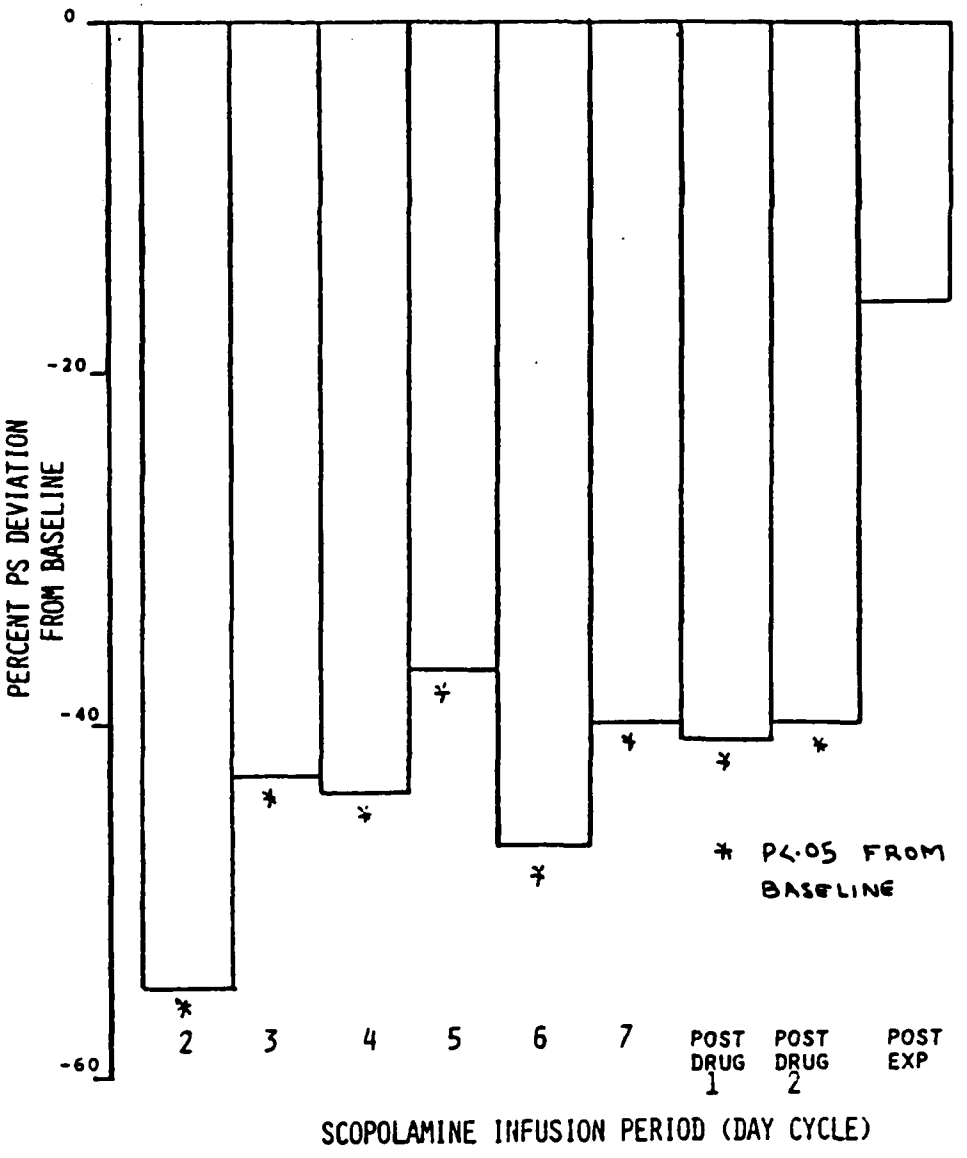

0
-20
-40
-60
PERCENT PS DEVIATION FROM BASELINE
2
3
4
5
6
7
POST DRUG 1
POST DRUG 2
POST EXP
* P<.05 FROM BASELINE
SCOPOLAMINE INFUSION PERIOD (DAY CYCLE)

Figure 18. PS change during the night cycle as a result of drug or saline infusions into the fourth ventricle.

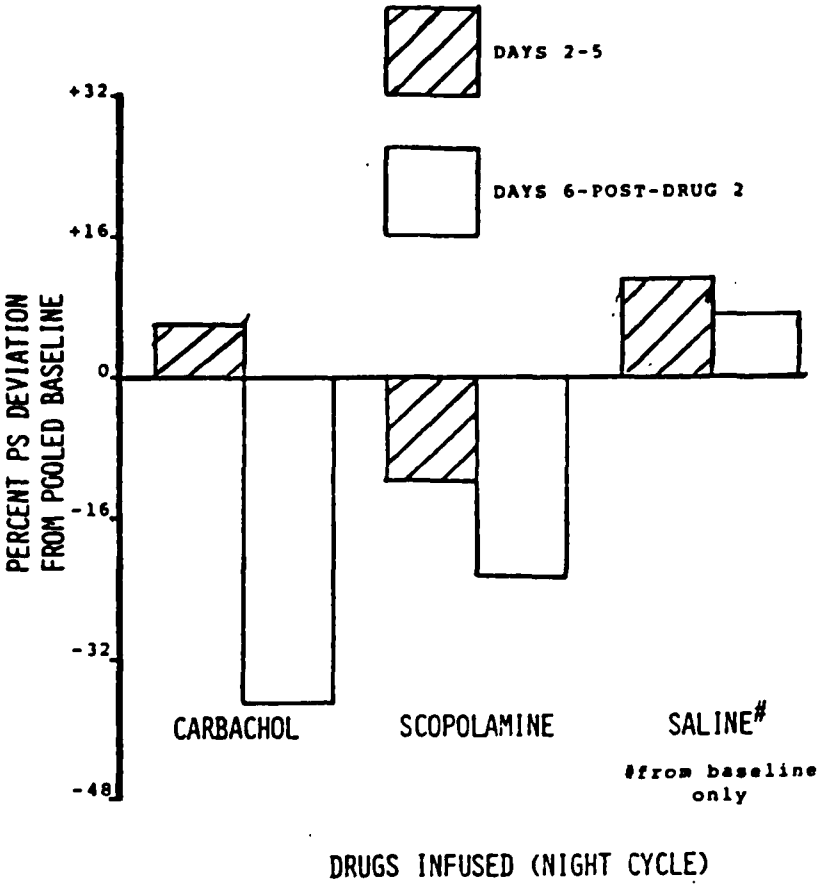

DAYS 2-5
DAYS 6-POST-DRUG 2
+32
+16
0
-16
-32
-48
PERCENT PS DEVIATION FROM POOLED BASELINE
CARBACHOL
SCOPOLAMINE
SALINE#
#from baseline only
DRUGS INFUSED (NIGHT CYCLE)

Figure 19. Comparision of the effect of carbachol infusions into the brainstem and the fourth ventricle (24 hr cycle).

Figure 19

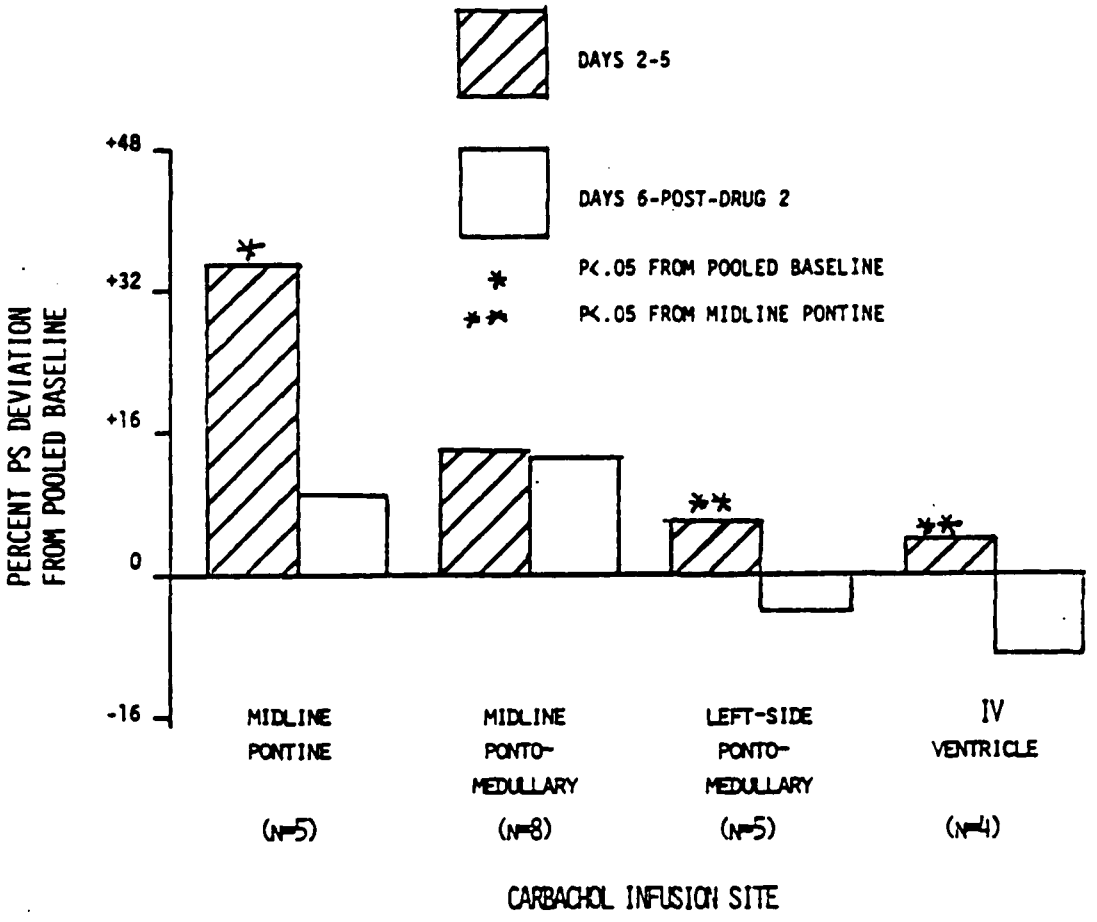

DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM POOLED BASELINE
** P<.05 FROM MIDLINE PONTINE
PERCENT PS DEVIATION FROM POOLED BASELINE
+48
+32
+16
0
-16
MIDLINE PONTINE (N=5)
MIDLINE PONTO-MEDULLARY (N=8)
LEFT-SIDE PONTO-MEDULLARY (N=5)
IV VENTRICLE (N=4)
CARBACHOL INFUSION SITE

Figure 20. Comparision of the effect of scopolamine infusions into the brainstem and the fourth ventricle (24 hr cycle).

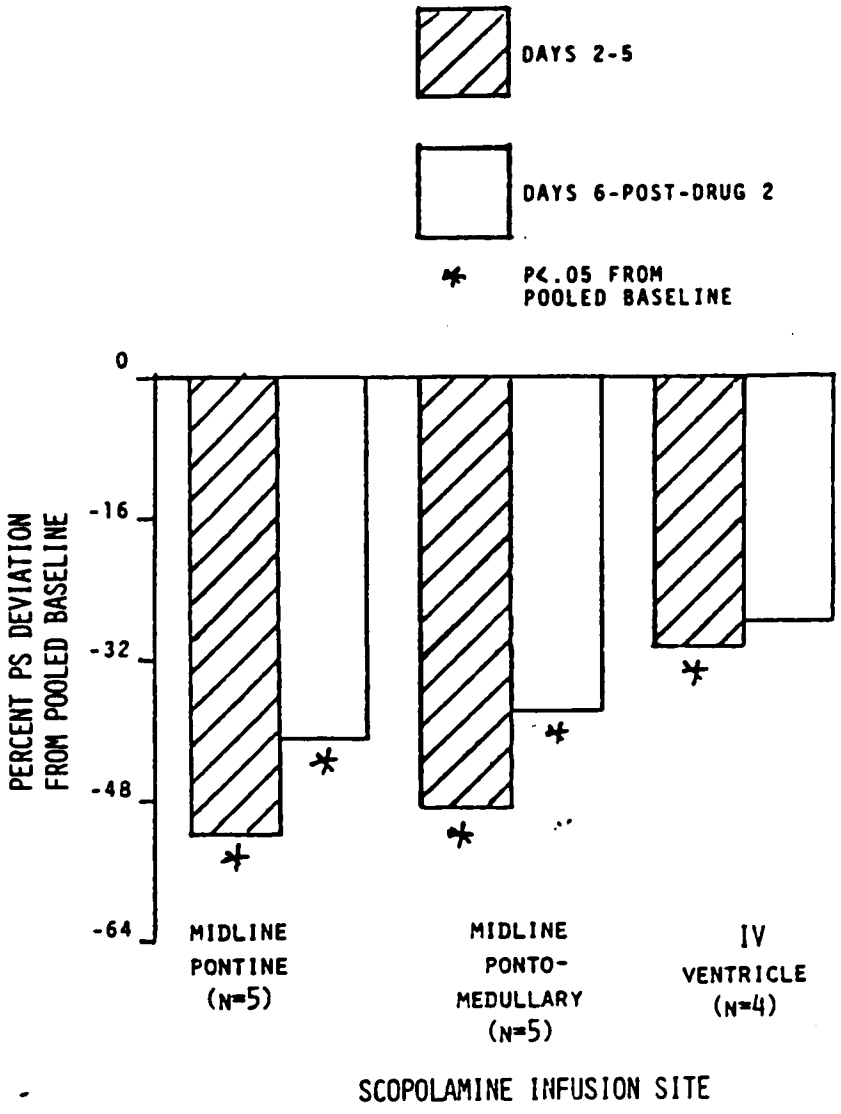

DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM POOLED BASELINE
PERCENT PS DEVIATION FROM POOLED BASELINE
0
-16
-32
-48
-64
MIDLINE PONTINE (N=5)
MIDLINE PONTO-MEDULLARY (N=5)
IV VENTRICLE (N=4)
SCOPOLAMINE INFUSION SITE

Figure 21. Comparision of the effect of saline infusion into brainstem and the fourth ventricle (24 hr cycle).

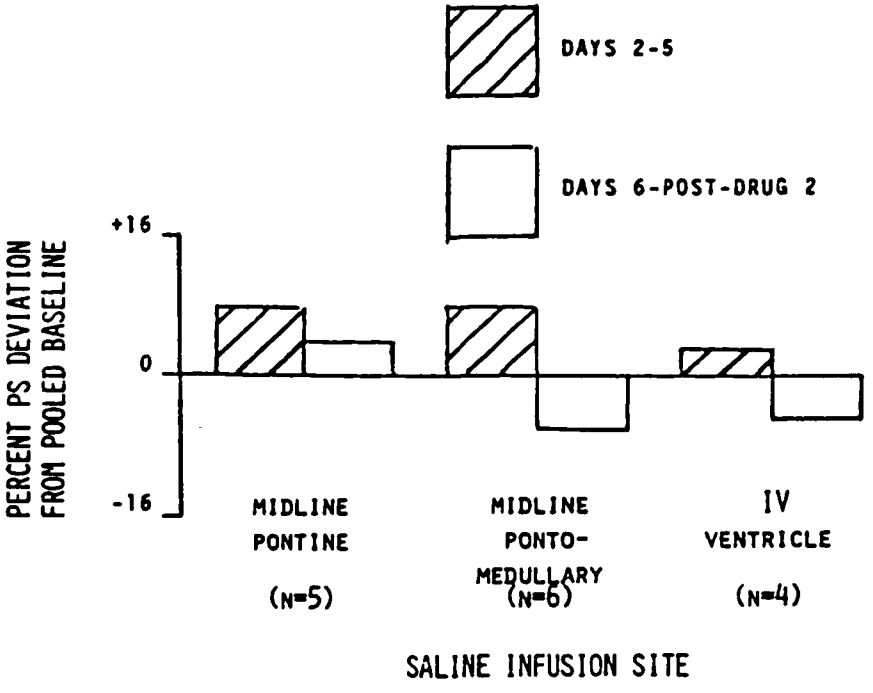

DAYS 2-5
DAYS 6-POST-DRUG 2
PERCENT PS DEVIATION FROM POOLED BASELINE
+16
0
-16
MIDLINE PONTINE
(N=5)
MIDLINE PONTO-MEDULLARY
(N=6)
IV VENTRICLE
(N=4)
SALINE INFUSION SITE

Figure 22. Comparision of the effect of carbachol infusions into the brainstem and the fourth ventricle - day cycle.

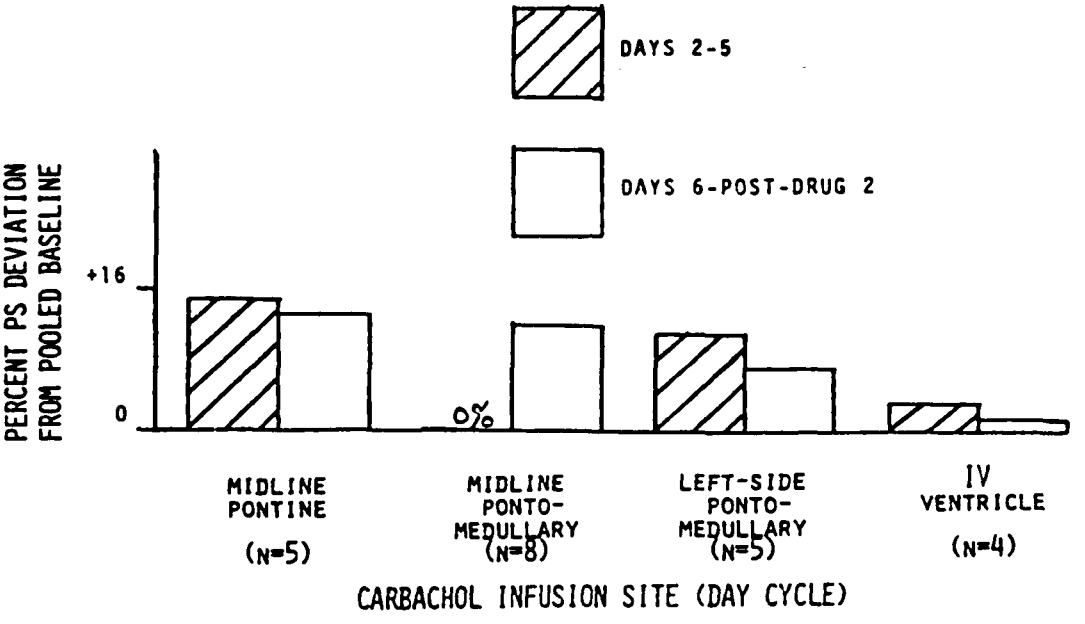

DAYS 2-5
DAYS 6-POST-DRUG 2
PERCENT PS DEVIATION FROM POOLED BASELINE
+16
0
0%
MIDLINE PONTINE (N=5)
MIDLINE PONTO-MEDULLARY (N=8)
LEFT-SIDE PONTO-MEDULLARY (N=5)
IV VENTRICLE (N=4)
CARBACHOL INFUSION SITE (DAY CYCLE)

Figure 23. Comparision of the effect of scopolamine infusions into the brainstem and the fourth ventricle - day cycle.

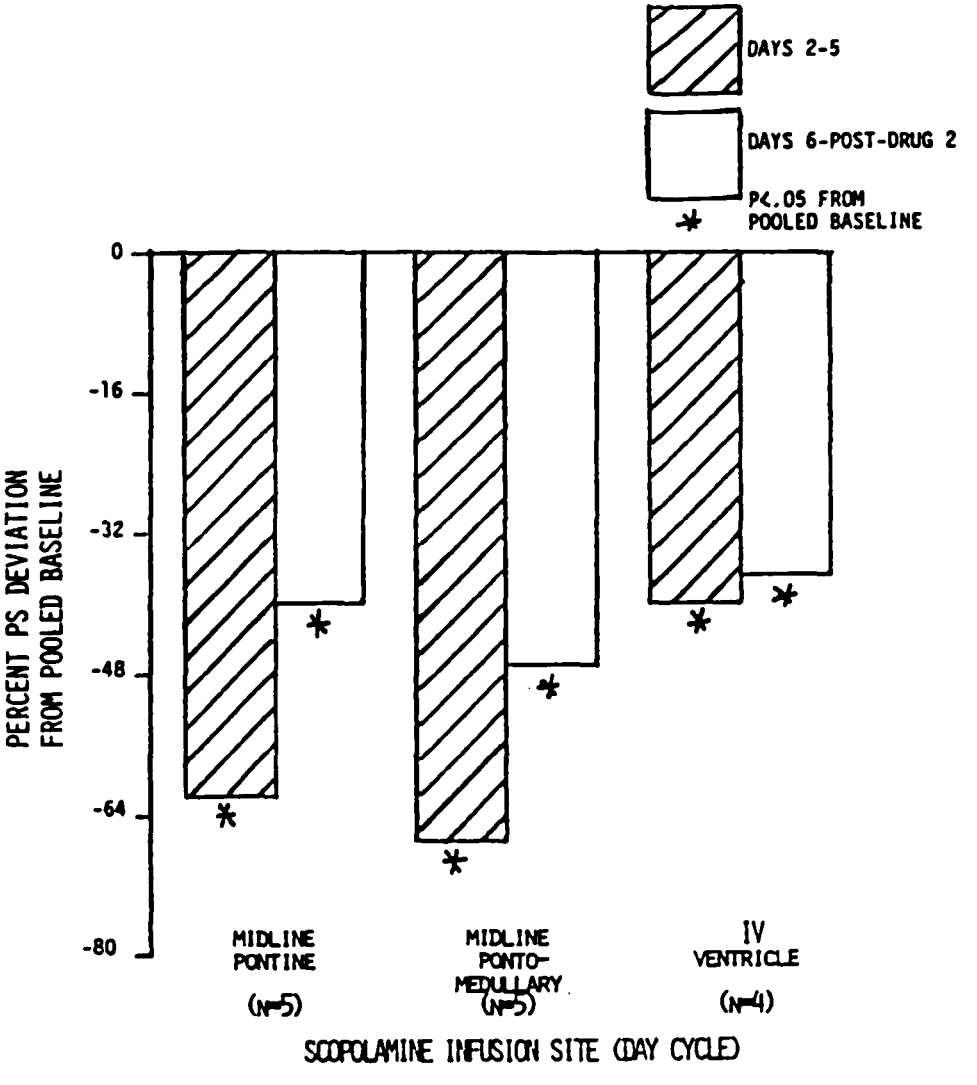

DAYS 2-5
DAYS 6-POST-DRUG 2
P<.05 FROM POOLED BASELINE
0
-16
-32
-48
-64
-80
PERCENT PS DEVIATION FROM POOLED BASELINE
MIDLINE PONTINE (N=5)
MIDLINE PONTO-MEDULLARY (N=5)
IV VENTRICLE (N=4)
SCOPOLAMINE INFUSION SITE (DAY CYCLE)

Figure 24. Comparision of the effect of saline infusions into the brainstem and the fourth ventricle - day cycle.

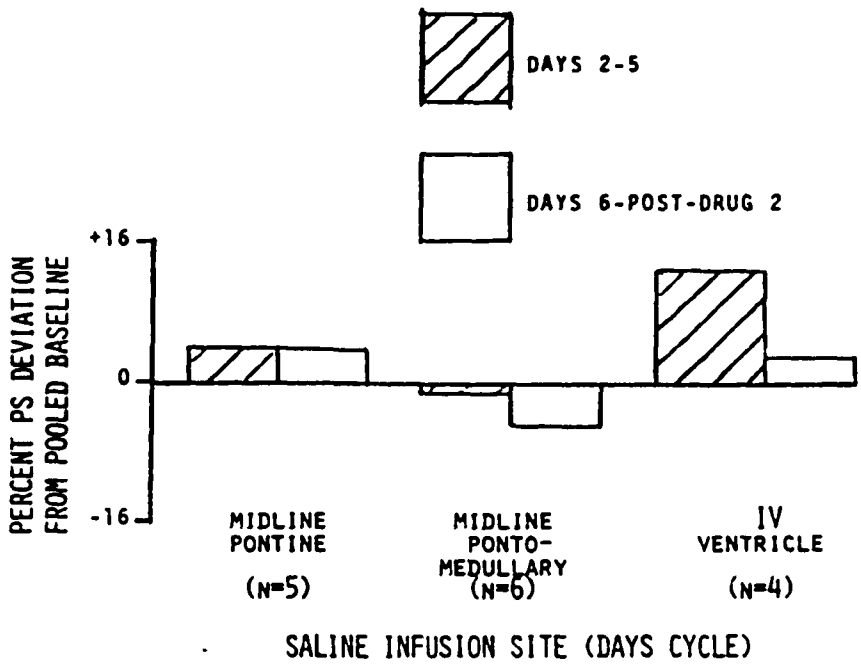

DAYS 2-5
DAYS 6-POST-DRUG 2
PERCENT PS DEVIATION FROM POOLED BASELINE
+16
0
-16
MIDLINE PONTINE
(N=5)
MIDLINE PONTO-MEDULLARY
(N=6)
IV VENTRICLE
(N=4)
SALINE INFUSION SITE (DAYS CYCLE)

Figure 25. Comparision of the effects of carbachol infusions into the brainstem and the fourth ventricle - night cycle.

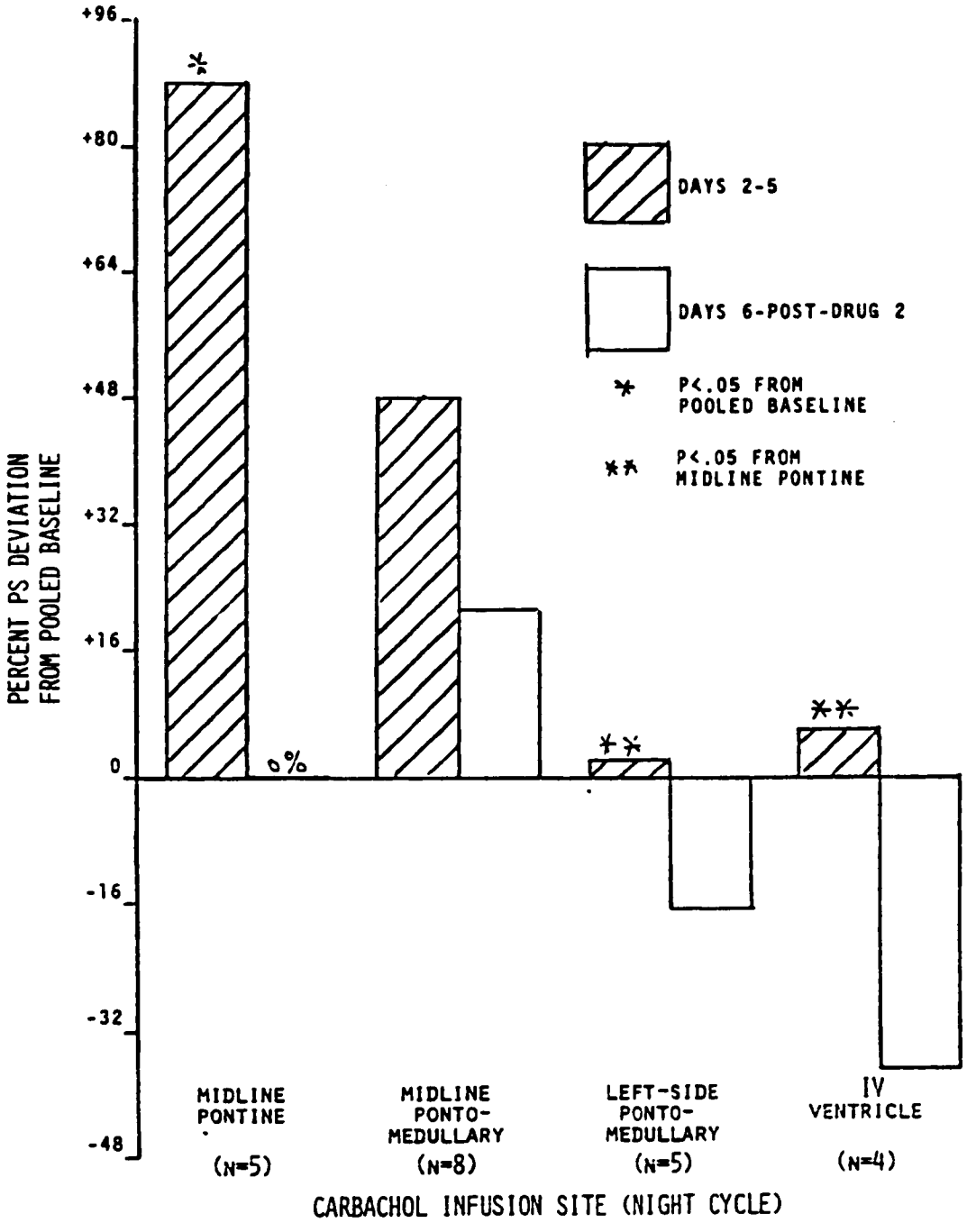

+96
+80
+64
+48
+32
+16
0
-16
-32
-48
PERCENT PS DEVIATION FROM POOLED BASELINE
DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM POOLED BASELINE
** P<.05 FROM MIDLINE PONTINE
0%
MIDLINE PONTINE (N=5)
MIDLINE PONTO-MEDULLARY (N=8)
LEFT-SIDE PONTO-MEDULLARY (N=5)
IV VENTRICLE (N=4)
CARBACHOL INFUSION SITE (NIGHT CYCLE)

Figure 26. Comparision of the effect of scopolamine infusions into the brainstem and the fourth ventricle - night cycle.

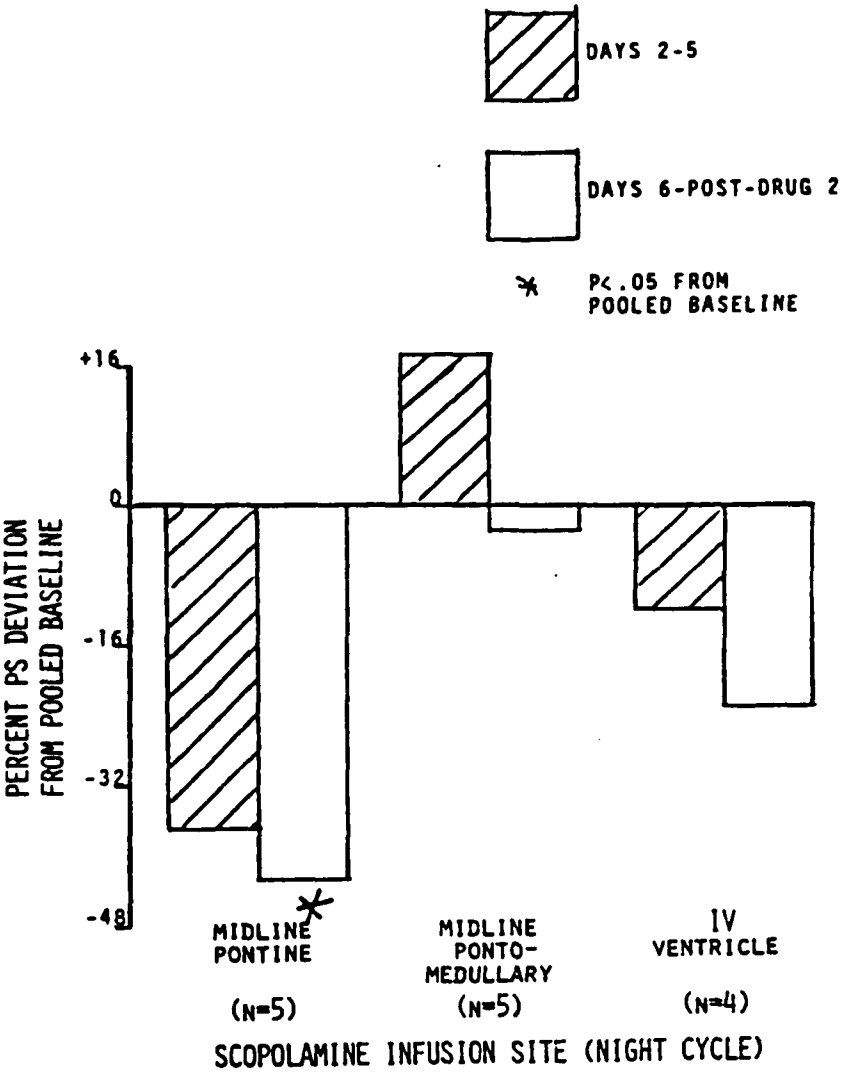

DAYS 2-5
DAYS 6-POST-DRUG 2
* P<.05 FROM POOLED BASELINE
+16
0
-16
-32
-48
PERCENT PS DEVIATION FROM POOLED BASELINE
*
MIDLINE PONTINE
(N=5)
MIDLINE PONTO-MEDULLARY
(N=5)
IV VENTRICLE
(N=4)
SCOPOLAMINE INFUSION SITE (NIGHT CYCLE)

Figure 27. Comparision of the effects of saline infusions into the brainstem and the fourth ventricle - night cycle.

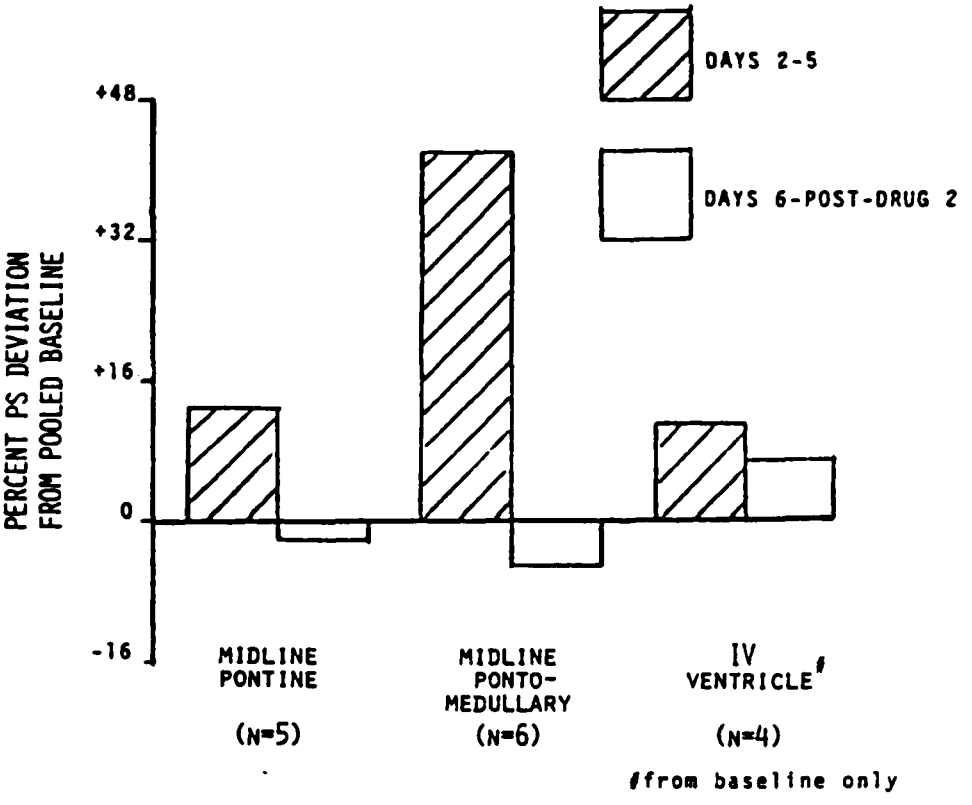

DAYS 2-5
DAYS 6-POST-DRUG 2
+48
+32
+16
0
-16
PERCENT PS DEVIATION FROM POOLED BASELINE
MIDLINE PONTINE
(N=5)
MIDLINE PONTO-MEDULLARY
(N=6)
IV VENTRICLE#
(N=4)
#from baseline only
SALINE INFUSION SITE (NIGHT CYCLE)

Figure 28. Schematized depiction of cannula placement in the 14 day pump infusion experiment. Coronal sections are adapted from Palkovits and Jacobowitz (1974).

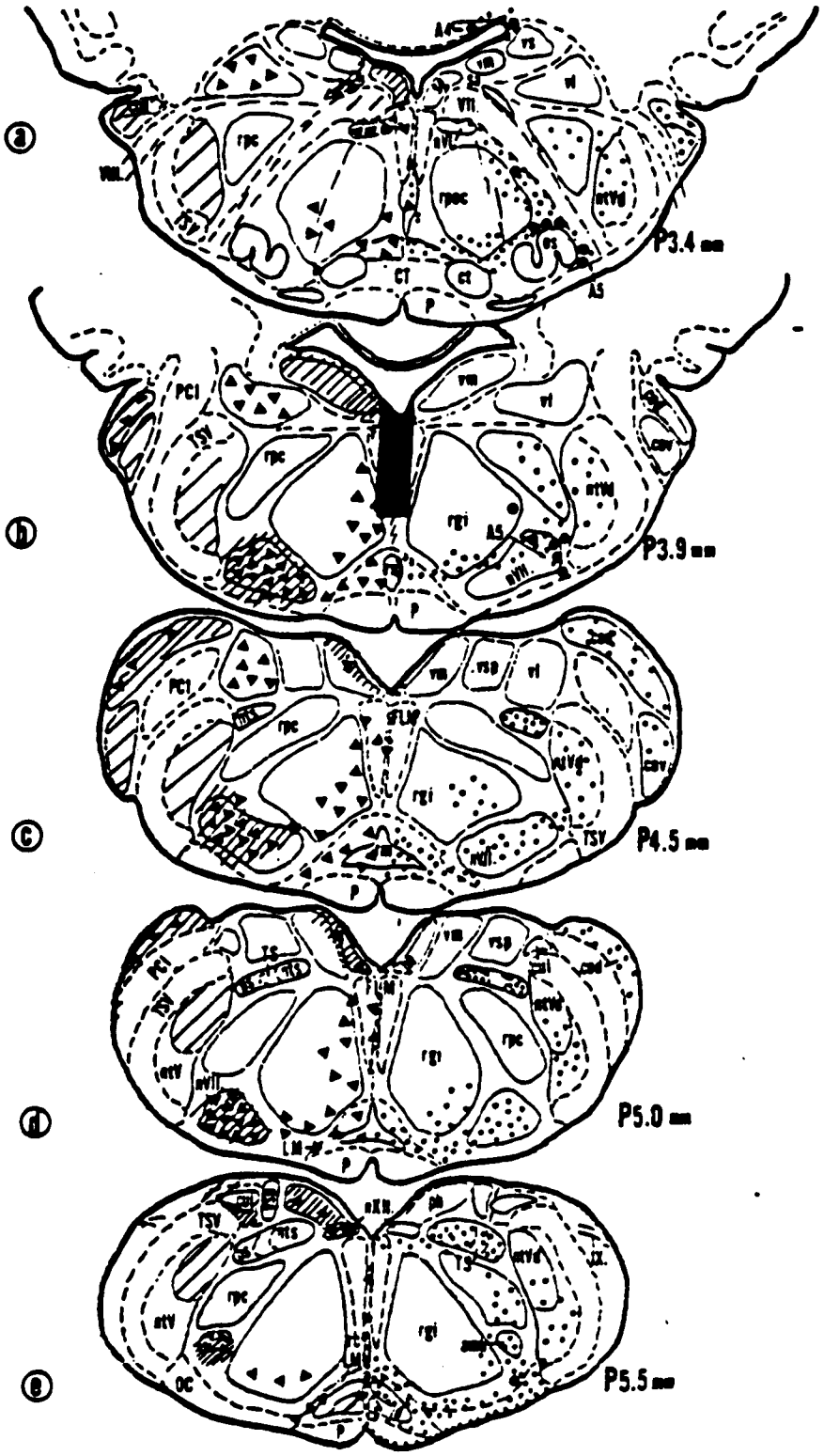

a
P3.4 mm
h
P3.9 mm
c
P4.5 mm
d
P5.0 mm
e
P5.5 mm

Figure 29. PS change as a result of 14 day infusions of carbachol. Pooled baseline refers to within group average of baseline and post-experimental baseline PS values.

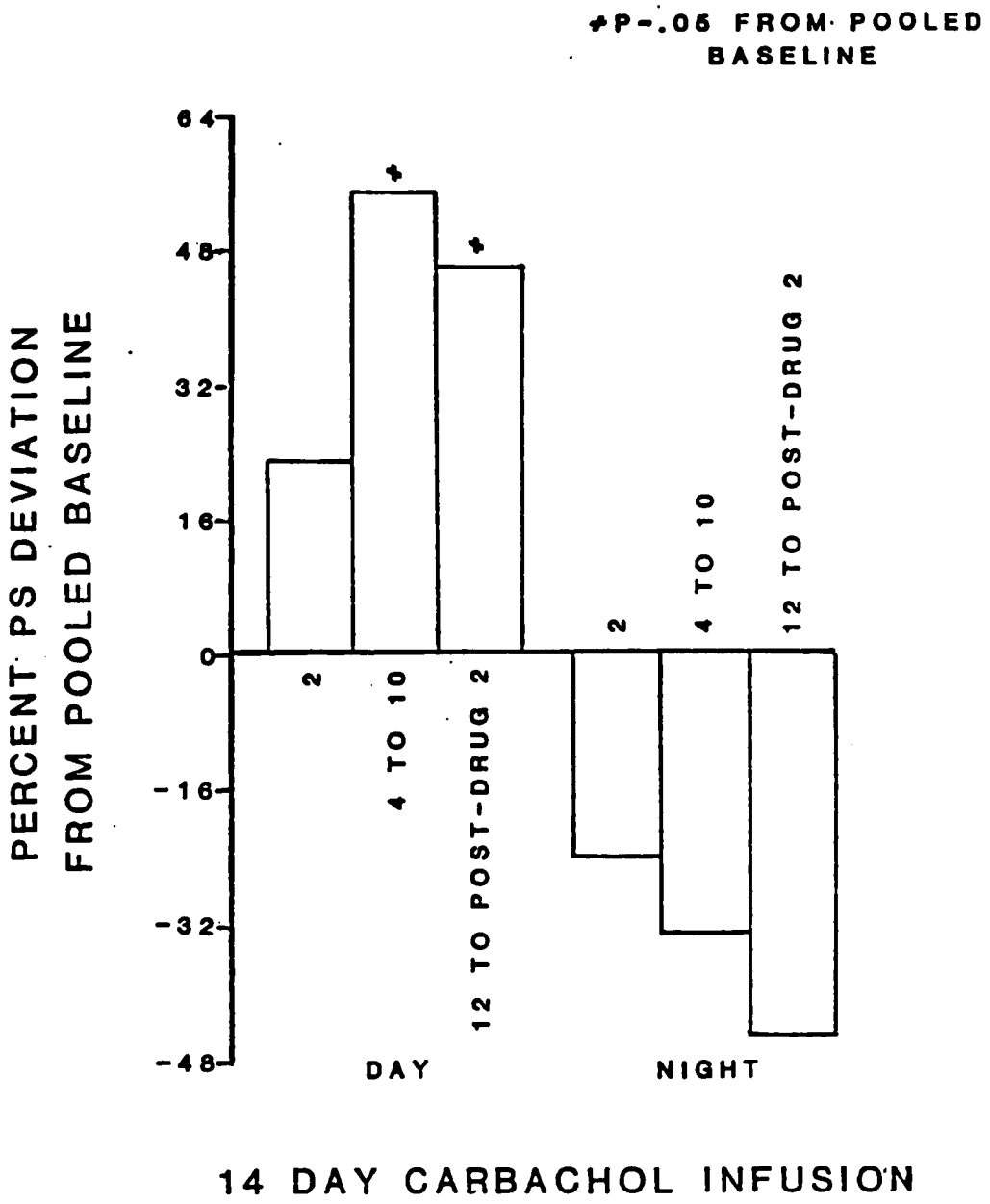

*P=.05 FROM POOLED BASELINE
PERCENT PS DEVIATION FROM POOLED BASELINE
64
48
32
16
0
-16
-32
-48
*
*
2
4 TO 10
12 TO POST-DRUG 2
2
4 TO 10
12 TO POST-DRUG 2
DAY
NIGHT
14 DAY CARBACHOL INFUSION

Figure 30. Percent change from training in avoidance scores during testing at 7 or 21 days. Independent groups of rats are implanted with pumps containing either carbachol, scopolamine or saline. Subsequently the animals are given 50 consecutive training trials in a two-way shuttlebox and then tested for retention 7 or 21 days later. For more details see Experiment 3, discussion.

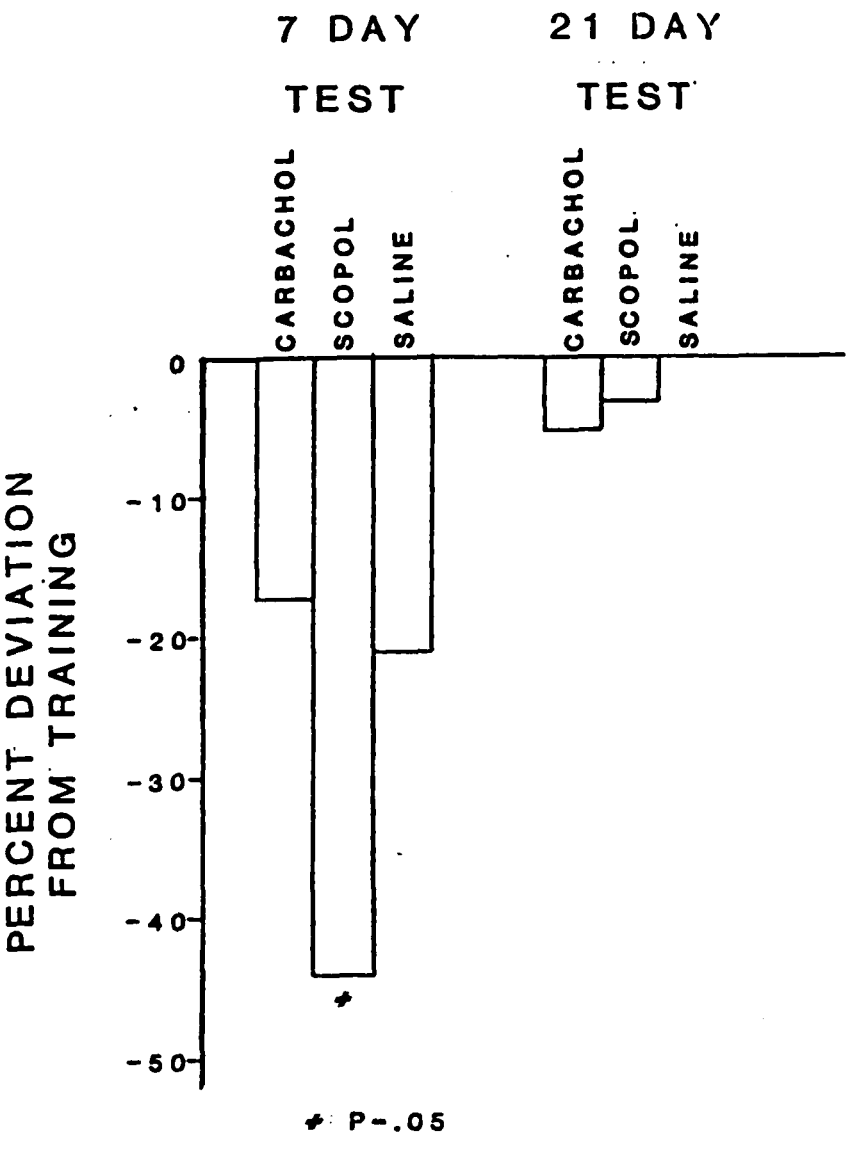
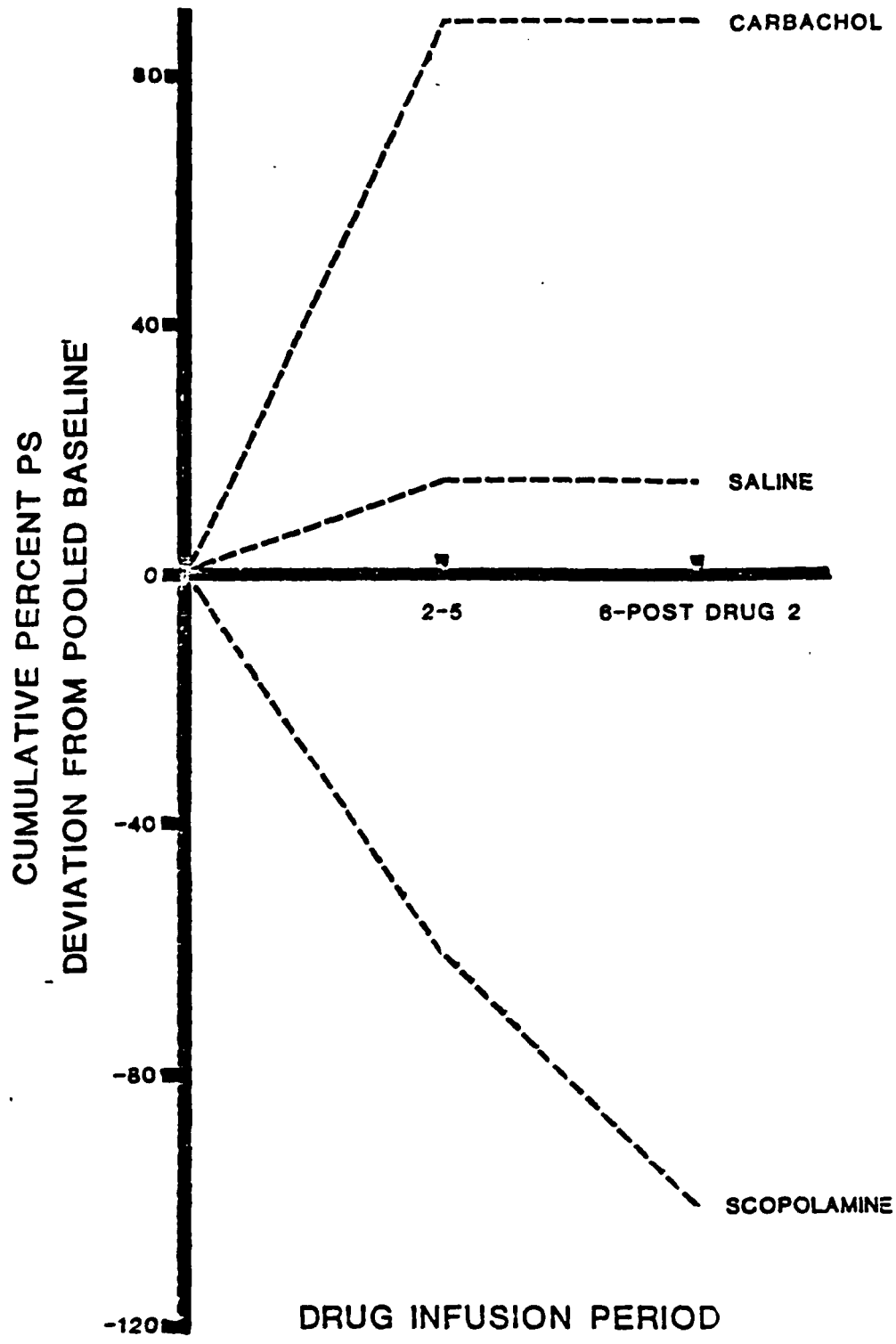

7 DAY TEST
21 DAY TEST
CARBACHOL
SCOPOL
SALINE
CARBACHOL
SCOPOL
SALINE
0
-10
-20
-30
-40
-50
PERCENT DEVIATION FROM TRAINING
* P=.05

Figure 31. Summary of the divergent PS alterations produced by carbachol, a muscarinic agonist, and scopolamine, a muscarinic receptor blocker.


CARBACHOL
SALINE
SCOPOLAMINE
80
40
0
-40
-80
-120
2-5
6-POST DRUG 2
CUMULATIVE PERCENT PS
DEVIATION FROM POOLED BASELINE
DRUG INFUSION PERIOD

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