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Electrical brain stimulation (EBS) of the periaqueductal gray (PAG) and the medial hypothalamus (MH) is known to serve as a discriminative and a negative reinforcing stimulus (NRS). Using a two-lever food reinforced discrimination paradigm and a switch-off paradigm, the present study investigated the effects of anxiolytic drugs and an anxiogenic drug on these stimulus effects. A prototypic anxiogenic, pentylenetetrazole (PTZ) potentiated both discriminative stimulus and NRS effects, whereas the full benzodiazepine (BZD) agonist diazepam (DZP), the partial BZD agonist abecarnil (ABC) and 5-HT_{1A} agonist buspirone (BUS, chronic regimen) attenuated a NRS effect. A BZD antagonist, flumazenil (FLU) blocked the effects of DZP and ABC on the NRS effects. DZP failed to attenuate the discriminative stimulus effect. Thus, present study extended the use of a switch-off paradigm to detect novel anxiolytic ABC (putative) and BUS as well as an anxiogenic PTZ. In addition, under the condition used in this study, the use of NRS in a switch-off paradigm more reliably detected both anxiolytic drugs and an anxiogenic drug than the use of discriminative stimulus in a two-lever food reinforced paradigm.

DISCRIMINATIVE AND NEGATIVE REINFORCING PROPERTIES

OF THE PERIAQUEDUCTAL GRAY AND

THE MEDIAL HYPOTHALAMUS

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ABBREVIATION

5-HT:	5-hydroxytryptamine
ABC:	abecarnil
BUS:	buspirone
BZD:	benzodiazepine
CDP:	chlordiazepoxide
DZP:	diazepam
EBS:	electrical brain stimulation
FLU:	flumazenil
MH:	medial hypothalamus
MLR:	mesencephalic locomotor region
NRS:	negative reinforcing stimulus
SOL:	switch-off latency
SOR:	switch-off response
PAG:	periaqueductal gray
PTZ:	pentylenetetrazole

CHAPTER I

INTRODUCTION

Statement of problem

Electrical brain stimulation (EBS) of certain brain areas has been known to produce a reinforcing stimulus. This stimulus can either be positive (Olds and Olds, 1962; Colpaert, et al., 1977; Druhan et al., 1987) or negative depending upon the brain areas stimulated. Among other structures, the dorsal periaqueductal gray (PAG) (Delgado 1954; Wada et al., 1970; Schenberg and Graeff, 1978; Audi and Graeff, 1984) and the medial hypothalamus (MH) (Atrens, 1973) have mostly been implicated in the negative reinforcing stimulus (NRS) induced by EBS. For instance, following stimulation of the PAG or the MH, a rat learns to press a lever to switch-off the stimulation (Audi and Graeff, 1984). This type of behavior is referred to as a switch-off response (SOR) and has been used as an index of the NRS produced by EBS (Delgado et al., 1954; Olds and Olds, 1962; Sandner et al., 1987; Depoortere et al., 1990a, b). One of the advantages of the switch-off paradigm over other behavioral paradigms is that the SOR can be accurately quantified by means of the switch-off

latency (SOL): the time elapsed between the onset and the offset of the stimulation by a press of a lever. A body of studies investigated the mechanisms that mediated the NRS as well as the drug effects on the responding induced by NRS, e.g. benzodiazepines (BZD) (Schenberg and Graeff, 1978; Graeff and Rawlins,1980; Depoortere et al., 1990a), a BZD receptor inverse agonist FG 7142 (Depoortere et al., 1990a), and serotonergic drugs (Kiser et al., 1978; Schenberg and Graeff, 1978). However, the present study is the first to investigate the effects of non-BZD drugs, abecarnil (ABC) and buspirone (BUS), and a prototypical anxiogenic drug, pentylenetetrazole (PTZ), on the responding induced by EBS. In addition, previous studies have not systematically differentiated drug effects on NRS from their non-specific motoric effects. To this end, the present study examined the possible drug-induced motoric disturbances using a high frequency stimulation paradigm. Since a high frequency stimulation facilitates SOR (Depoortere et al., 1990b), if a drug disturbs an animal's motoric capacity, SOR will no longer be facilitated even at a high frequency stimulation.

EBS serves not only as a NRS but also as a discriminative stimulus in the control of an animal's behavior. Typically, a group of animals are trained to discriminate the presence or the absence of EBS. If they make an appropriate response, i.e. choose a stimulation-associated lever in the

presence of EBS, they get a reinforcer. Discrimination studies using EBS have been carried out to demonstrate discriminative properties of positive reinforcing brain areas such as the dorsal raphe (Hirschhorn et al., 1975), lateral hypothalamus (Colpaert, 1977a,b; Stutz and Maroli, 1978) and ventral tegmental area (Druhan et al., 1987). It has only recently been established that EBS of negative reinforcing brain areas such as the PAG (Jenck et al., 1986) and the MH (Lappuke et al., 1982) also can produce a discriminative stimulus following EBS. In these studies (Jenck et al., 1986; Lappuke et al., 1982), a motivating stimulus for a rat to perform an operant task was a NRS. Thus, it was not clear whether a discriminative stimulus can be differentiated from a NRS. Subsequently, Depoortere et al. (1990c) employed a two-lever food-reinforced paradigm and attempted to differentiate the discriminative stimulus effects from the NRS effects of the EBS of the mesencephalic locomotor region (MLR). In their study, a food-reinforcer was used to motivate a rat to perform a discriminative task. In this context, the present study investigated whether a two-lever food-reinforced paradigm could be extended to detect the discriminative properties of the PAG or the MH stimulation. Furthermore, this study examined the pharmacological effects of DZP and PTZ on those effects of the EBS of these brain areas.

EBS

Electrical change is a fundamental feature of the flow of information between and within neurons. As a consequence, functions of the brain are exceedingly sensitive to imposed electrical fluctuations which pass through the surface of the neurons (Doty,1969). Early investigators utilized this fact to demonstrate that EBS of certain brain areas can serve as a reinforcing stimulus: a positive or a negative reinforcing stimulus (Olds and Olds, 1962; Colpaert, et al., 1977; Druhan et al., 1987; Wada and Matsuda 1970). A positive reinforcing stimulus is defined as a stimulus which by being given to the environment strengthens and maintains an animal's behavior (Campbell, 1955). In contrast, a NRS is a stimulus which by being taken out of the environment strengthens and maintains an animal's behavior (Campbell, 1955). For example, EBS of the lateral hypothalamus (Olds and Olds, 1962) and ventral tegmental area (Colpaert, et al., 1977; Druhan et al., 1987) have been implicated to produce a positive reinforcing stimulus because animals stimulated at these areas press a lever to continue receiving EBS.

A NRS is originally inferred from the observation that stimulation of certain brain areas elicited a defense-like response defined as a response of an animal to threatening or stressful stimuli (Graeff, 1988).

The brain structures that induce defense-like behaviors following EBS extend from dorsomedial parts of the amygdala (Mecican and Delgado, 1953) along the stria terminalis to the level of the anterior commissure (Fernandez de Molina and Hunsperger, 1959) and into the preoptic area, the MH (Flynn, 1967), and the PAG (Graeff, 1988). In rats, typical defense-like behaviors induced by EBS of the dorsal part of the PAG are freezing, running, jumping, escape behavior, biting and vocalization (Brandao et al., 1982; Fardin et al., 1984). In cats, vocalization, threatening postures, flight reaction and rageful attack have been elicited by EBS of the PAG and the MH (Flynn, 1967; Mecican and Delgado, 1953; Fernandez de Molina and Hunsperger, 1962). These behaviors are abolished when neurons in the PAG and adjacent tegmentum of rats and cats are destroyed by a lesion (Edward and Adams, 1974; Halpern 1968; Liebman et al., 1970; Skultety, 1963).

In the beginning of this line of research, studies simply demonstrated that an animal substituted the defense-like behaviors by an operant task such as a press of a lever to interrupt the stimulation. Subsequently, this stimulation was referred to as a NRS because the animal maintained responding to terminate the stimulation (Campbell, 1955; Wada et al., 1970; Schenberg and Graeff, 1978). The significance of this finding was

that a variety of behavioral effects produced by EBS could now be measured with objective techniques.

In 1954, Delgado et al. found that cats stimulated in the superior part of the tectal area, the lateral nuclear mass of the thalamus, and the inferiomedial part of the hippocampal gyrus learned to rotate a wheel to turn off the EBS. Similar experiments performed by Wada et al. (1970) showed that cats stimulated at the PAG or the MH learned to push a plate attached to a window of a box to terminate the EBS. Thus, the PAG or the MH stimulations were demonstrated to be negatively reinforcing.

Instead of terminating the EBS at once, subjects were also taught to decrease the strength of the stimulus in small steps. This method was called the titration method. Boren and Jerry (1961) performed experiments in which rhesus monkeys stimulated at the midbrain reticular formation learned to press a lever to reduce the current intensity of the stimulation. In the absence of a response, the current rose in small steps until the animal initiated responding again. This method was later used by Kiser et al., (1978) to test drug effects on stimulus-induced responding.

Olds and Olds (1962) used a negative reinforcing brain stimulation in combination with a positive reinforcing brain stimulation to investigate the interactions between the two opposite stimuli. The rats were implanted at both the tegmental escape-producing areas and the hypothalamic

approach-producing areas. The positive reinforcing effect was tested by presenting a lateral hypothalamic stimulus after each lever press, while a stimulation was being applied to the negative reinforcing area in the tegmentum. The negative reinforcing effect was tested by presenting repeated tegmental stimulations and interrupting these stimulations for four seconds after each lever response. Simultaneously, a stimulation was applied to the positive reinfocing area in the lateral hypothalamus. The negative stimulus impeded the positively reinforced behavior. The positive stimulus, however, was far less effective in impeding the escape response. They concluded that there was a one-way inhibition from the tegmental escape areas to the lateral hypothalamic positive reinforcing areas.

The negative reinforcing effects of MH stimulation were demonstrated using a shuttle box technique (Atrens, 1973). Each rat implanted at the MH or the lateral hypothalamus was placed in a shuttle box. Inside the box, moving to one end interrupted a photobeam, which consequently initiated an EBS. This EBS was terminated only by interrupting another photobeam transecting the chamber from the opposite end. The latencies to initiate and to terminate the EBS were recorded as indices of a positive and a negative reinforcing stimulus, respectively. Although the animals both initiated and terminated the MH stimulation, the NRS was the major

component of this stimulation because the latency to terminate it was significantly shorter than the latency to initiate it. In addition, the latency to initiate the MH stimulation was significantly longer than that of lateral hypothalamus. In contrast, the latency to terminate the MH stimulation was significantly shorter than that of the lateral hypothalamus stimulation. These data suggested that the MH stimulation produced strong NRS effects, whereas the lateral hypothalamus stimulation produced strong positive reinforcing effects. This is consistent with the result from another study in which, when each press stimulated the medial as well as the lateral hypothalamus simultaneously, the self-stimulation of the lateral hypothalamus was stopped. Furthermore, the rate of lateral hypothalamic self-stimulation was increased after destruction of the MH (Hoebel and Teitelbaum, 1962).

Using a switch-off paradigm, Schmitt, et al. (1979) proposed a functional relationship between two negative reinforcing stimuli, the PAG and the MH stimulation. They showed that PAG lesions produced a significant decrease in the SOR induced by MH stimulation.

Sandner et al. (1987) attempted to map the PAG subdivisions with respect to the stimulation-elicited overt behaviors as well as SOR. They observed that jumps were most often elicited by the dorsal and rostral PAG stimulation, whereas squeals and rearings were most frequently

elicited from the stimulation in the caudal and the subareas surrounding the aqueduct, respectively. With increasing stimulation strengths, the SOL of the dorsal PAG stimulation shortened faster than the SOL of the ventrally applied stimulations. In addition, they found that the switch-off learning was not systematically generalized from a given PAG site to another. The authors suggested that functional subdivisions existed within the PAG.

Recently, Depoortere et al. (1990b) demonstrated that rats stimulated at the mesencephalic locomotor region (MLR) learned to switch-off the stimulation, suggesting that the stimulation is negatively reinforcing. Hence, the negative reinforcing brain structures have been proposed to include the MLR.

Effects of BZDs on the responding induced by the negative reinforcing brain stimulation

BZDs are among the drugs that are most frequently used to examine the drug effects on the defense-like behaviors as well as on operant behaviors induced by negative reinforcing brain stimulation. Mallick (1970) tested the behavioral effects of BZDs following the EBS of the perifornical area of the anterior hypothalamus in cats. EBS of this area elicited hissing, a defense-like response in cats. The stimulation intensity

that produced three consecutive hissing responses 30 seconds apart was measured as the hissing threshold. DZP (4 and 7.5 mg/kg), CDP (10 and 15 mg/kg), oxazepam (12 mg/kg) and phenobarbital (40 mg/kg) significantly raised the hissing threshold. In agreement, Panksepp (1971) reported a similar effect of CDP (10 mg/kg i.p.) in rats on the defensive aggression elicited by EBS of the MH: mouse-attack elicited by stimulation of the MH was abolished following CDP pretreatment.

A defense-like response is characterized not only by behavioral aspects but also by autonomic changes (Abrahams et al., 1960), e.g. increase in cardiac output, heart rate and blood pressure. Haefely et al. (1981) examined the effects of the BZDs on autonomic changes induced by EBS. In cats and rats, rises in the blood pressure and the heart rate induced by the EBS of the PAG were selectively decreased by several BZDs and barbiturates when given i.v. or i.p.. Schenberg and Graeff (1978) obtained similar results in anesthetized rats. In these animals, microinjection of midazolam (40 and 160 nmol) into the dorsal PAG attenuated the hypertension, tachycardia and hyperventilation produced by EBS.

Brandao et al. (1982) examined the behavioral effects induced by both BZDs microinjected into the dorsal PAG and dorsal PAG stimulation together. Rats were placed inside a shuttle box and electrically

stimulated with increasing current intensities until they started to run back and forth. Flight behavior was measured by the number of times the animals crossed the dividing line while running from one compartment of the shuttle-box to the other. Microinjection of CDP (160 and 320 nmol/l) significantly raised the threshold current intensity to induce this flight behavior.

Schenberg and Graeff (1978) measured the effects of CDP on the SOR elicited by the PAG stimulation by means of SOL. They found that CDP (3 and 10 mg/kg, i.p.) increased the SOL to terminate the EBS. Since these doses of CDP caused only moderate sedation or ataxia, this effect was interpreted as being due to the attenuation of the NRS rather than being due to a non-specific behavioral suppression. This effect of CDP on NRS is consistent with the results from another experiment by Graeff and Rawlins (1980). Lever pressing behaviors maintained by food or water reinforcers, but suppressed by brief EBS applied at the dorsal PAG, were markedly increased by CDP (5-17 mg/kg, i.p.). In contrast, psychoactive drugs from the psychostimulant, antipsychotic, antidepressant or anticonvulsant classes either did not change or decreased the escape threshold.

The above studies provide evidence that BZDs attenuate an animals' response to negatively reinforcing brain stimulation. Subsequently, these

data led to the hypothesis that GABA/BZD receptors modulate the NRS induced by EBS.

GABA/BZD receptor in modulation of NRS

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter whose receptor constitutes a supramolecular receptor complex with the BZD receptor, the barbiturate receptor and the chloride channel (Fahn, 1976). The theory has been proposed that the BZDs act by potentiating the coupling between the GABA receptor and the chloride channel (Cooper et al., 1986). There are at least two types of GABA receptors, namely the $GABA_A$ and the $GABA_B$ receptors. $GABA_A$ receptors are stimulated by muscimol, THIP [4,5,6,7-tetrahydroisoxazolo (5,4-C) pyridine-3-ol] and isoguvacine and antagonized competitively by bicuculline and non-competitively by picrotoxin. The $GABA_A$ receptor is coupled to the chloride channel and appears to be linked to the BZD receptor. GABA acts at both $GABA_A$ and $GABA_B$ receptors (Simmonds, 1983). The effect of GABA on $GABA_A$ receptors is enhanced by BZD agonists and reduced by BZD inverse agonists (Haefely et al., 1981). In contrast, the $GABA_B$ receptor is associated with calcium and is neither activated by $GABA_A$ agonists (muscimol) nor blocked by $GABA_A$

antagonists (bicuculline and picrotoxin). They are, however, selectively stimulated by p-chlorophenylalanine (baclofen), which is devoid of any action on $GABA_A$ receptors (Hill and Bowery, 1981; Simmonds, 1983).

The PAG and the MH are rich in GABA (Fahn, 1976). Even though regional variations in the GABA content exist in the MH, there is less evidence for such variations within the PAG (Kimura and Kuriyama, 1975). The following results indicate that GABA/BZD receptors are involved in the modulation of the NRS induced by EBS: 1) Operant escape induced by PAG stimulation was suppressed by enhanced GABAergic neurotransmission (Bovier et al., 1982). In rats stimulated in the PAG, GABA agonists, progabide (100 mg/kg, i.p.) and DZP (5 and 7 mg/kg, i.p.), increased the latency as well as the current thresholds to escape to a safe compartment. Blockade of GABA receptors by bicuculine reduced the action of progabide and DZP. 2) The microinjections of $GABA_A$ agonists muscimol, isoguvacine and THIP into the PAG raised the current threshold to switch-off the PAG stimulation (Audi and Graeff, 1987). Whenever the rat crossed the midline separating the two compartments of the shuttle box, this switched-off the EBS. On the other hand, $GABA_B$ agonist baclofen did not affect the threshold. 3) Conversely, intense flight, locomotor activity, running and

jumping out of the box were induced by microinjections of $GABA_A$ antagonists, bicuculine or picrotoxin into the PAG (Brandao et al., 1982; Scala et al., 1982). Similar reactions were observed by microinjections of glutamate decarboxylase (semicarbazide), inhibitors of GABA synthesis into the PAG (Brandao et al., 1986). The microinjection of SR 95103, a new $GABA_A$ receptor blocker into the MH or the PAG also produced similar behavioral responses (Schmitt, et al., 1985). Since these escape behaviors were antagonized by a pretreatment with THIP or midazolam (Audi and Graeff, 1987), it was suggested that these behaviors were the expression of a NRS state rather than non-specific motoric responses.

Depoortere et al. (1990a) have characterized the effects of CDP on the SOR induced by EBS of the PAG or the MLR. Whereas CDP (5 mg/kg i.p.) increased the SOL for the PAG stimulation, CDP did not affect the SOL for MLR stimulation. They suggested that even though the EBS of both structures induced SOR, the differential negative reinforcing properties existed between the PAG and the MLR stimulations.

Haefely et al. (1979) investigated the effects of ethanol on the escape response of the rat. A rat implanted with an electrode at the dorsal PAG was placed in a box with a barrier dividing the cage in half. EBS was maintained until the rat escaped to the other side of the cage or until a maximum EBS time had elapsed. Pretreatment with ethanol (0.2-1.6 g/kg,

oral) increased both the escape latency and the current threshold (Bovier et al., 1983). Ethanol is believed to facilitate the coupling of the GABA receptor and the chloride channel (Haefely et al., 1985). Thus, these data also provide evidence of GABA/BZD modulation in negative reinforcing effect of brain stimulation. Taken together, these results strengthen the hypothesis that the GABA/BZD receptor complex plays a role to modulate this negative reinforcing brain system.

Abecarnil, a novel putative anxiolytic

ABC is a β-carboline-3-carboxylic acid ester derivative with partial agonist properties that show a high affinity for central BZD receptors (Ballenger et al., 1991). BZDs are effective anxiolytics, but their side effect profiles and, most importantly, their liability to induce dependence following chronic administration has led to a clinical need for novel anxiolytics possessing the effectiveness of the BZDs without their unwanted side effects. Consequently, compounds such as abecarnil (ABC) that produce fewer side effects than the BZDs were introduced. Like the full agonist, DZP, ABC possesses anxiolytic and anti-convulsant activities (Turski,1990), but it has considerably less muscle relaxant and sedative effects (Ballenger et al., 1991) as well as less liability of dependence (Emmett-Oglesby et al.,1993) than DZP.

Regarding the behavioral effects of ABC, so far, no study has examined its effects on animals' responding induced by EBS. However, there are data in punishment and conflict procedures from which the behavioral effects of ABC can be inferred. A punishment procedure is defined as the presentation of a stimulus which reduces the probability of responding (Iversen, 1984). In a conflict procedure, the subject experiences a conflict between the urge to make a response to get a reinforcer and the presentation of a punisher if they do so (Iversen, 1984).

Stephens et al. (1990) systematically tested the anxiolytic activity of ABC in rats and mice. In the four-plate test (Stephens and Kehr, 1985), mice were placed in the center of a rectangular chamber with a floor divided into four metal plates. After 20 sec during which they were allowed to explore freely, the mice received a mild brief shock each time they crossed from one plate to another. This electric shock functions as a punisher resulting in a decrease of the animal's response. The increased number of crossings from one plate to another following ABC treatment was taken as an indice of its anxiolytic activity. ABC significantly increased punished locomotor activity (number of crossings) of mice at doses of 0.39 mg/kg i.p. compared to the dose of DZP of 0.78 mg/kg, i.p..

In agreement, ABC at a dose 5-fold less than DZP increased the mean number of licks in the waterlick conflict test (Peterson and Buus,

1981). Rats were water-deprived for 48 hr before they were allowed to have access to a water tube. Each 20th lick was punished with an electric shock to the tongue. The anxiolytic effect of ABC was measured from the minimal effective dose producing a significant difference between the pre- and the post-drug punished responding.

The anxiolytic effect of ABC was also shown in a study of the PTZ discriminative stimulus (the PTZ discriminative stimulus reflects the anxiogenic properties of drugs, Shearman and Lal, 1978). Rats were trained to discriminate PTZ (15 mg/kg i. p.) from saline in a standard two-lever operant drug discrimination task (Shearman and Lal, 1978). Responding was maintained by food available on a FR10 (fixed-ratio 10: subjects get a reinforcer by appropriate ten respondings) schedule of reinforcement. The anxiolytic effects of ABC were assessed following i.p. administration 15 min before PTZ treatment, and the discrimination task was then observed. ABC antagonized the PTZ discriminative cue at a dose ten times lower than that of DZP.

In the Cook-Davidson (1973) conflict test, after rats were trained to operate a lever to obtain food, every 10th press was reinforced with both food and shock. There was a decrease in the number of responding in a control group, whereas ABC (10-20 mg/kg) enhanced punished-lever pressing (Stephens et al., 1990).

The above data show that ABC is effective as an anxiolytic in these behavioral paradigms with a greater potency than DZP. To this end, the present study investigated the behavioral effects of ABC on the responding maintained by negative reinforcing PAG stimulation. In addition, its potency was compared to that of DZP in a switch-off paradigm.

Serotonergic modulation of NRS

5-HT (5-Hydroxytryptamine) is a monoaminergic neurotransmitter implicated in EBS-induced behavior. The behavioral effects of 5-HT following EBS of the PAG (Kiser et al., 1978; Schutz et al., 1985), MH (Leroux and Myers, 1975), dorsal midbrain tegmentum (Kiser and Lebovitz, 1974) and the ventral reticular formation (Kiser et al., 1978) have been reported. 5-HT containing neurons are known to be restricted to clusters of cells lying in or near the midline or raphe regions of the pons and the upper brain stem (Cooper et al., 1986). From these cell groups major projections ascend to the forebrain (Iversen, 1984).

Studies to investigate the behavioral effects of 5-HT following negative reinforcing brain stimulation have only recently been established. This line of study was originated from the observation that after intraventricular infusion, 5-HT selectively accumulated in the mid

brain regions where EBS produces NRS in animals, (Wise et al., 1972). Among multiple 5-HT receptors existing in brain tissue, 5-HT_1 and 5-HT_2 subtype receptors have been examined in negative reinforcing brain stimulation (Patel and Slater, 1984).

In general, reducing 5-HT neurotransmission facilitates responding to terminate negative reinforcing brain stimulation. In contrast, enhancing 5-HT neurotransmission inhibits this response. For instance, lever-pressing switch-off response induced by EBS of the PAG was facilitated by 5-HT synthesis inhibitor para-chlorophenylalanine (Kiser et al., 1978), or by 5-HT receptor blockers methysergide and cyproheptadine (Schenberg and Graeff 1978). These facilitated responses were inhibited by 5-HT synthesis precursor 5-hydroxytryptophan (5-HTP), or by presynaptic 5-HT uptake inhibitor, chlorimipramine (Kiser et al., 1978).

More direct evidence was obtained using chemitrodes which allowed both microinjection of serotonin and simultaneous EBS of the PAG in the rat. 5-HT (5, 10, and 20 nmol) and the direct 5-HT receptor agonist, 5-methoxy-N,N-dimethyltryptamine (0.5, 1 and 2 nmol) were microinjected into the PAG of rats chronically implanted with chemitrodes. Both drugs raised the lowest current intensity (escape threshold) which induced escape inside a shuttle box. This increased threshold was decreased by either metergoline (a non-selective 5-HT blocker) or ketanserin (a

selective 5-HT_2 blocker) previously injected into the PAG (Schutz et al., 1985). In addition, the selective 5-HT uptake inhibitor, zimelidine, raised the escape threshold following its microinjection into the dorsal PAG (Schutz et al., 1985).

The inhibitory role of 5-HT transmission in negative reinforcing brain stimulation extended to other brain structures as well. Microinjection of 5-HT increased the escape threshold in rats stimulated at the MH (Leroux and Myers, 1975). The 5-HT depleting drug para-chlorophenylalanine produced a marked increase in bar pressing response to decrease the stimulation current of EBS of the dorsal midbrain tegmentum (Kiser and Lebovitz, 1975).

Kiser et al. (1978) investigated the effects of a 5-HT-depleting drug, PCPA, on lever pressing responses produced by the EBS of the dorsal PAG or the ventral reticular formation. Rats stimulated in the dorsal PAG or the ventral reticular formation learned to press a lever to progressively decrease the stimulation current. Following preteatment with PCPA, the dorsal PAG group showed a marked increase in the rate of response whereas the ventral reticular formation group showed no change. These results indicate that reducing 5-HT neurotransmission affects the escape behavior induced by EBS of one brain structure, but not the other. These

authors suggested that the negative reinforcing brain stimulation might be mediated by more than one neural system.

The involvement of 5-HT in this line of research has been extended to the 5-HT_1 receptor subtypes level (Verge et al., 1985; Jenck et al., 1989). Effects of 8-OH-DPAT, a selective 5-HT_{1A} agonist on PAG stimulation was compared with those of mCPP, preferentially active on the 5-HT_{1B} and /or 5-HT_{1C} receptor (Jenck et al., 1989). Opposite effects were found with these two agonists: 8-OH-DPAT dose dependently decreased the threshold for EBS-induced escape behavior while mCPP dose dependently increased the threshold.

However, there are doubts that the modulation of 5-HT of the negatively reinforcing brain stimulation is due to a specific effect on NRS rather than merely a general motor depression. This argument comes from the finding that 5-OH-tryptophan causes a general suppression of behavior in the pigeon and 5-HT, infused into the ventricles, depresses self-stimulation in the rat (Wise et. al., 1972). Gerson and Baldessarini (1980) have suggested that the projection of 5-HT neurons located in the raphe region to forebrain substantia nigra appears to inhibit general motor behavior. Their study showed that lesions of 5-HT input to the substantia nigra enhances behavior which is dependent on dopamine release

(Carter and Pycock, 1979). However, no experiments systematically examined these controversial data. Furthermore, this controversy reflects the complex nature of 5-HT involvement in mechanisms underlying the response to NRS of the EBS or the various behavioral models.

Buspirone

Buspirone (BUS) is a novel non-BZD anxiolytic drug with an efficacy and a potency comparable to DZP (Taylor et al., 1985). There are a number of pharmacological differences between BUS and BZD anxiolytics. The most striking difference is that BUS lacks the anticonvulsant, sedative, and muscle relaxant effects of the BZDs (Allen et al., 1974; Goldberg and Finnerty, 1979). In addition, buspirone is clinically reported to show its anti-anxiety effects only after chronic treatment of 10-14 days.

BUS is extensively metabolized to yield a major metabolite, 1(2-pyrimidinyl) piperazine (1-PP). 1-PP tends to accumulate in the brain, particularly when the drug is given orally (Geller and Hartmann, 1982). There are suggestions that the pharmacological effects of BUS may be mediated, at least partially, through 1-PP (Geller and Hartmann, 1982).

Regarding the mechanism of action of BUS, there are different hypotheses. One hypothesis is that BUS acts at presynaptic 5-HT_{1A}

receptors particularly in the raphe nuclei, resulting in the suppression of 5-HT neurotransmission (Mennini et al., 1986). There are data supporting this hypothesis. Thus, Meller et al. (1990) observed a large 5-HT_{1A} receptor reservoir in the raphe nuclei. Acute administration of BUS led to a reduction of 5-HIAA (a metabolite of 5-HT) concentrations in hippocampus (Mennini et al., 1986) and in the hypothalamus (Hjorth and Carlsson, 1982). Mennini et al. (1986) suggested that the modification of this turnover was attributed to the activation of 5-HT_{1A} receptors.

However, another hypothesis was that the anxiolytic actions of BUS cannot be explained solely on the basis of its affinity for 5-HT_{1A} receptors, and that the anxiolytic effect of BUS share a mode of action with BZD. Garattini et al. (1983) reported the possible involvement of the GABA/BZD system in the anxiolytic effects of BUS because BUS increased the binding of GABA to washed brain membranes of rats. Using autoradiography, one study showed that chronic treatment with BUS induced adaptive changes of GABA/BZD receptors in the substantia nigra (Gobbi et al., 1991). Goeders et al. (1987) investigated *in vivo* biding of [^{3}H] Ro 15-1788 on BZD receptors following BUS administration. While BUS did not affect the binding of [^{3}H] Ro 15-1788 to the BZD receptors *in vitro* in cerebellum, medulla and cortex, significant dose-related increases

were observed for the *in vivo* binding of [^{3}H] Ro 15-1788 to those BZD receptors in mice .

Another problem is that the behavioral effects of BUS are active in some models, but not in other models. For instance, BUS showed its activity in the following experiments. BUS inhibited responding of rats trained to jump a barrier to avoid electric shock (Riblet et al., 1982). BUS also inhibited foot shock-induced fighting in the mouse (Geller and Blum, 1970). BUS produced taming effects in aggressive rhesus monkeys whose aggression was measured by the animals' responses to prodding with a pole (Tompkins et al., 1980). In this test, DZP was active but elicited both hypoactivity and ataxia whereas BUS showed no ataxia. BUS has also been shown to be active in both rat and monkey conflict procedures employing shock-induced suppression of feeding behavior (Geller and Hartmann, 1982).

In contrast, in punishment procedures, several studies have failed to find an anxiolytic effect of BUS in rodents and nonhuman primates (Goldberg and Finnerty, 1979; Wada and Fukuda, 1991). For instance, the effects of DZP and a new anxiolytic, DN-2327, were compared to that of BUS in rats in the elevated plus-maze test (Wada and Fukuda, 1991). In an elevated plus-maze with two open arms and two closed arms, the percentage of time spent on the open arms and percentage of open-arm

entries are measured as an index of anxiolytic effects. DZP and DN-2327 dose-dependently increased two indices. However, BUS (2.5-20 mg/kg p.o.) did not affect either of these responses. These controversial data bring attention to the view of Iversen (1984) that punishment procedures and conflict tests measure impulsive behavior of animals rather than the nature of anxiety.

To this end, the present study investigated whether BUS attenuates animals' responses induced by EBS of the PAG as BZD drugs did in a switch-off paradigm. In addition, the acute and chronic effects of BUS were also examined based on the previous data that BUS produce differential effects depending upon acute or chronic regimen (Gobbi et al., 1990).

PTZ

Based on the evidence that BZD anxiolytic drugs attenuate negative reinforcing brain stimulation, it seems plausible that an anxiogenic drug may possibly potentiate the NRS effect. Although the potentiation of that stimulus effect have not been studied systematically, there is a report of drug-induced potentiation of NRS. Depoortere et. al. (1990b) found that the BZD receptor partial inverse agonist, FG 7142 (2.5, 5 and 10 mg/kg i.p) decreased the SOL of PAG stimulation. They suggested that the

potentiating effect of this drug is specific, and not the consequence of a drug-induced general excitatory effect since FG 7142 treatment did not decrease the SOL of MLR stimulation.

The present study selected PTZ to examine whether PTZ would potentiate the discriminative and negative reinforcing effects induced by EBS of the PAG or the MH. PTZ is a prototype anxiogenic drug which produces intense anxiety in humans (Rodin, 1958; Rodin and Calhoun, 1970) and fulfills the criteria for studying anxiogenic effects (Lal and Emmett-Oglesby, 1983). Lal and co-workers (Lal and Fielding, 1979; Shearman and Lal, 1979) proposed measuring the anxiogenic effects of drugs using drug discrimination. Since then, PTZ has been studied in a drug discrimination paradigm and reported to produce interoceptive [those originating within the body (Lal and Emmett-Oglesby, 1983)] discriminative stimuli (Lal and Shearman, 1980). Typically, in PTZ discrimination experiments (Lal and Shearman, 1980), rats are trained to press one lever for a reinforcer after an injection of PTZ and the other lever for a reinforcer after injection of saline. The first ten responses on the correct lever results in delivery of one food pellet (FR10). When acquisition of such response differentiation is reliably established, the drug is said to produce a discriminative stimulus that controls the differential responding in trained rats (Shearman and Lal, 1979). A series

of studies examined the effects of BZDs or barbiturates to antagonize PTZ-induced discriminative stimulus. CDP, DZP (Shearman and Lal, 1979), flurazepam, midazolam (Benjamin et al., 1987), phenobarbital and meprobamate (Wilson and Bennet, 1989) antagonized the stimulus. However, no study has employed PTZ to examine its effect on animals' responding induced by negative reinforcing brain stimulation. Thus, the present study investigated the effects of PTZ on SOR as well as on the discriminative stimulus induced by PAG stimulation.

Switch-off paradigm

The switch-off paradigm employed in this study is an operant task in which rats press a bar (switch-off response) in order to interrupt EBS (Delgado et al., 1954; Olds and Olds, 1962; Sandner et al., 1987). In a body of experiments, the switch-off test has been used to detect a NRS induced by EBS. The latency to switch-off the EBS (switch-off latency, SOL) is defined as the time elapsed between the onset of EBS and the press of a lever, which switch-off the EBS (Sandner et al., 1987). SOL is inversely related to the strength of the EBS, i.e., the greater the strength of the EBS, the shorter the latency (Depoortere et al., 1990a). One of the advantages of the switch-off paradigm over other behavioral paradigms is that switch-off behaviors can be accurately quantified by means of the

SOL. Most previous studies which employed this paradigm tested BZD drugs such as DZP, CDP, oxazepam and midazolam, and serotonergic drugs. However, the present study is the first time the switch-off paradigm has been employed to study the novel anxiolytics, ABC (putative anxiolytic) and BUS, and a prototypic anxiogenic PTZ.

Discriminative properties of EBS

EBS of certain brain areas serves not only as a NRS but also as a discriminative stimulus in the control of an animal's behavior. Typically, a group of animals are trained to discriminate the presence or the absence of EBS. If they make an appropriate response, i.e. choose a stimulation-associated lever in the presence of EBS, they get food or water as a reinforcement. Their discrimination responding under both conditions are evaluated in the presence of a third manipulation, such as systemic administration of a drug. The stimuli produced by brain stimulation and systemic drug administration differ since brain stimulation affects a limited number of anatomical sites and the consequences of brain stimulation may be expected to be short-lived (Deutch, 1964). Nevertheless, the cue produced by brain stimulation is considered procedurally interchangeable with that produced by systemic administration of drugs (Hayes and Mayer,

1987). This is in part because both stimuli are considered as interoceptive stimuli: stimuli originating within the body (Lal, 1979).

Animals stimulated at the dorsal raphe (Hirschhorn et al., 1975), lateral hypothalamus (Colpaert, 1977a,b; Stutz and Maroli, 1978) and ventral tegmental area (Druhan et al., 1987a,b) learn not only to self-stimulate but also to discriminate the presence and absence of EBS. Thus, these brain structures are known to have positive reinforcing as well as discriminative properties. It was only recently that a discrimination paradigm was established in negative reinforcing brain areas such as the PAG (Jenck et al., 1986), MH (Lappuke et al., 1982) and MLR (Depoortere et al., 1990).

Lappuke et al. (1982) investigated whether animals can discriminate the two brain stimulations applied to different brain structures: the PAG and the MH. Rats learned to press a lever in order to switch-off a stimulation applied to the MH or to the dorsal PAG. Once the SOR was acquired, each rat was placed in a T maze, consisting of a start box and two arms. Stimulation in the PAG or the MH were delivered when the rat was in the start box. The rat was then allowed to leave the start box and to switch-off the PAG stimulation by pressing a lever located in one arm of the maze and to switch-off the MH stimulation by pressing a lever located in the other arm. The rat still discriminated between the two sites when

various stimulation intensities or frequencies were randomly applied to either site. Lappuke et al. (1982) suggested that although both sites of stimulation elicited the SOR, stimulation of each site provides a different stimulus cue.

The above study utilized negative reinforcing brain stimulation itself to motivate rats to discriminate the stimulus. Thus, it was not clear whether the motivating stimulus is a NRS or a discriminative stimulus. Subsequently, Depoortere et al. (1990c) used food reinforcers rather than EBS to motivate rats to discriminate EBS by employing a two-lever food-reinforced paradigm. Since a food-reinforcer serves as a positive reinforcing stimulus, the latter method may provide a more distinct stimulus dissociated from the NRS than the former. In that experiment (Depoortere et al., 1990c), rats were trained for food reward to press one lever in the presence of EBS of the MLR and the other lever in the absence of EBS. It was found that rats acquired a discriminative responding to the MLR stimulation, hence the EBS of the MLR had discriminative properties. In addition, the number of rats selecting the EBS-lever increased as the intensity and frequency of EBS increased. Since EBS of the MLR was proposed to serve as a NRS (Depoortere et al., 1990), Depoortere et al. (1990c) suggested that the negative

reinforcing properties of the MLR stimulation might constitute a component of the discriminative cue induced by MLR stimulation.

The present study investigated if a two-lever food-reinforced discrimination paradigm could be extended to detect the discriminative properties EBS of the PAG and the MH. Furthermore, demonstration of the negatively reinforcing properties of EBS of the PAG and the MH in the discriminative paradigm was attempted using DZP, a prototypical anxiolytic drug, and PTZ, a prototypical anxiogenic drug, on the discriminative properties of these brain structures. As proposed by Depoortere et al. (1990c), if the discriminative cue is based on the negative nature of the reinforcing brain stimulation, it subsequently gives rise to the following questions. Can DZP treatment attenuate the discriminative cue of PAG and of MH stimulation? Can PTZ produce the opposite effect to that of DZP on this stimulus effect? The later question is based on the reports that BZDs antagonized the discriminative cue of PTZ as described above.

Taken together, if an anxiolytic attenuates and an anxiogenic potentiates the stimulus effect, it can be assumed that the stimulus is analogous to the human anxiety in this animal model. Thus, in the present study, the discrimination paradigm is used to examine the

discriminative properties of EBS in a manner similar to that used for the PTZ discrimination paradigm.

Experimental rationale

Using the switch-off paradigm, the present study was undertaken to investigate whether a NRS induced by the EBS of the PAG could be extended to detect novel anxiolytics ABC (putative anxiolytic), BUS and a prototypic anxiogenic, PTZ. The combined effects of DZP or ABC with the BZD antagonist FLU on the SOR were also analyzed to investigate the involvement of GABA/BZD receptors in modulation of a NRS effect. In order to examine if the effects of these drugs on SOR is influenced by motoric disturbance, SOR following drug pretreatments were retested at a higher frequency. This test is based on the following assumptions: SOR is facilitated at high frequency (Depoortere et al., 1990a). If drugs disturb an animal's motoric capacity, SOR would be no longer facilitated even at a high frequency.

In discrimination paradigm using a two-lever food-reinforced procedure, this study investigated whether rats can discriminate the presence or the absence of brain stimulation of the PAG and the MH. Pharmacological tests were then conducted with PTZ and DZP. The hypotheses for this study in both paradigms are such that negative reinforcing and the discriminative properties of the EBS of the PAG and

the MH would be attenuated by the pretreatments with anxiolytics DZP, ABC and BUS and potentiated by an anxiogenic, PTZ.

CHAPTER II

MATERIALS AND METHODS

Animals

Male Long-Evans rats (23 rats for the discrimination experiment and 15 rats for switch-off experiment) (Harlan Sprague Dawley, Indianapolis, IN) were housed singly and kept on a 12 h light/dark cycle (light on between 8:00 a.m. and 8:00 p.m.). Weights of the rats were maintained at 330 g $\pm$ 10 g (discrimination experiment) or 400 $\pm$ 10 g (switch-off experiment) by restricting access to food pellets (Harlan Teklad Medicine, WC). For all rats, food was freely available three days before and three days after surgery.

Apparatus

For both the discrimination and switch-off experiments (Figure 1), rats were trained and tested in 8 standard operant chambers (model E-10-10TC, Coulbourn Instruments, Lehigh Valley, PA) fitted with two levers (5 cm above the floor) and a house-light. Food pellets (45 mg, BioServ, Frenchtown, NJ) were dispensed by a Lehigh Valley Electronics food pellet dispenser via a trough located equidistant from the two levers.

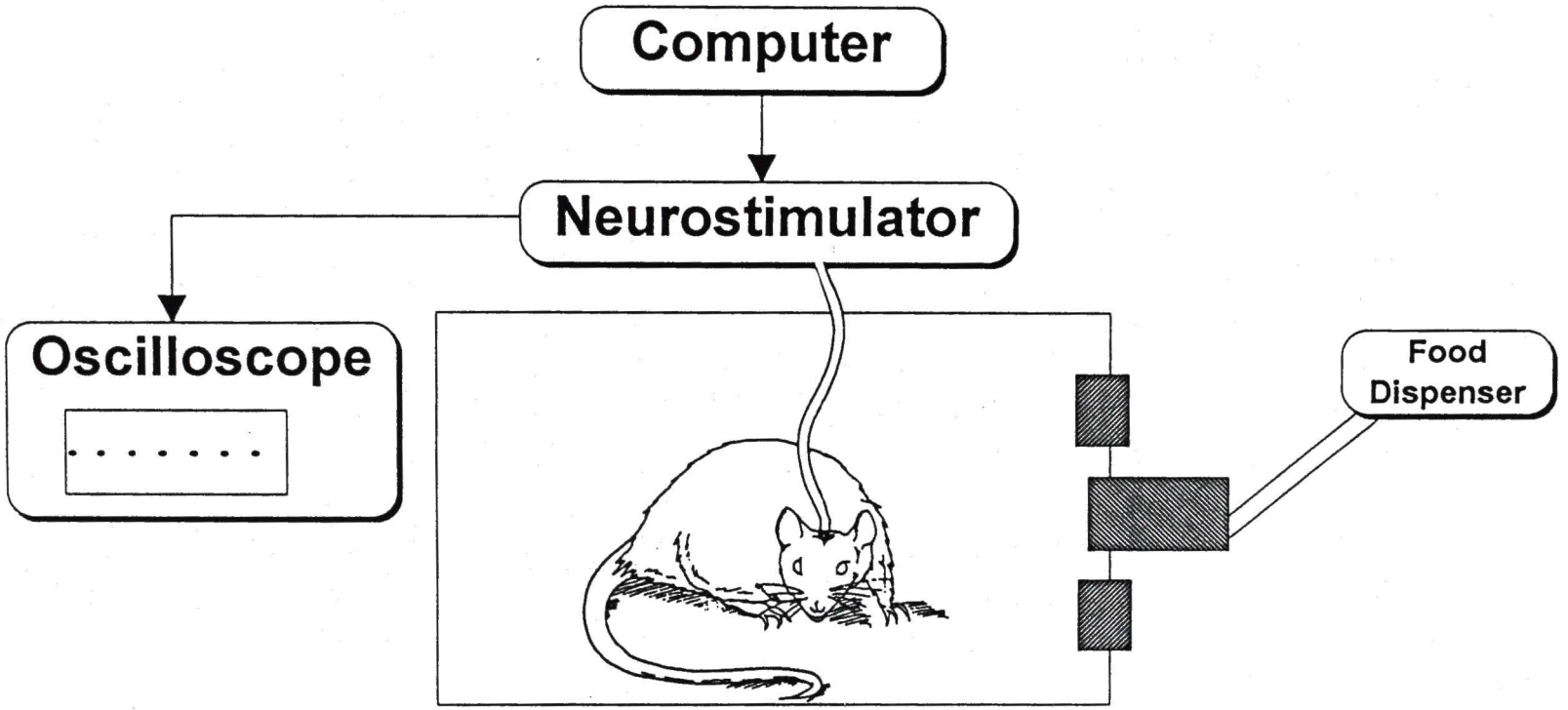


Figure 1. Schematic diagram of electrical brain stimulation apparatus.

Each chamber was enclosed in a ventilated and sound-attenuated cubicle, and was connected to an IBM PC compatible computer via an interface (LVB, Med Associates Georgia, VT). All events were recorded and controlled by the "Operant Package for Neurosciences Software" (Spencer and Emmett-Oglesby, 1985). Electrical brain stimulation was delivered by optically-isolated pulse neurostimulators (model 2100, A-M Systems, Everett, WA) through a 5-lead spring-shielded cable suspended from the ceiling of the operant box. Each rat was connected to the stimulation circuit by means of a 5-lead cable terminated by a miniature 5-pin male connector. Electrical parameters were continuously monitored for each neurostimulator on an oscilloscope (Tektronics, Everett, WA) across a 100-kohms resistor in series with the stimulation circuit.

Lever press shaping

Lever press shaping was performed for the discrimination experiment only. After two weeks of isolation, rats were trained to press a lever under a fixed-ratio 1 schedule (FR1: one reinforcer after one lever press). After lever-pressing was acquired, the schedule of reinforcement was increased to a fixed-ratio two (FR2: one reinforcer after two lever presses), with the delivery of each reinforcer followed by a 30-sec time-out period in the dark. Pressing the lever during this time-out period had no consequences.

Rats were required to accumulate at least 75% (shaping criterion, Depoortere et al., 1990c) of their total presses on any lever when the stimulus

lights were on in order to be selected for the EBS discrimination training. The reason for this criterion is because a discrimination paradigm is based on a animal's preference to select one lever over the other lever. Rats that had reached this criterion were suspended from training and given food ad lib for three days. On the fourth day, they were implanted with stimulation electrodes.

Surgery

Rats were injected with atropine (1 mg/kg, s.c.) followed by a mixture of ketamine (100 mg/kg), chlordiazepoxide (20 mg/kg) and nalbuphine (10 mg/kg). After induction of anesthesia, they were placed in a stereotaxic frame in the flat skull position. One electrode was implanted in the periaqueductal gray (PAG), another electrode was implanted on the opposite side in the medial hypothalamus (MH). Half of the rats were implanted with PAG (MH) electrodes in the right (left) side, the other half with the PAG (MH) electrode in the left (right) side. All PAG electrodes were implanted with a 15 degree mediolateral angle in order to avoid piercing the sagittal venous sinus. The following stereotaxic coordinates were used for the PAG: anterioposterial (AP): 1 mm, mediolateral (ML): 1.7 mm, DV (dorsoven-tral): 5.5 mm. For the MH the coordinates were AP: 4.5 mm, ML: 0.3 mm, DV: 9.5 mm. For both brain structures, all three coordinates are given with respect to lambda.

Electrodes were made of two 175 μm stainless steel Teflon-coated threads (A-M Systems, Everett, WA), twisted and held together with a cyanoacrylate glue (superglue, Rawn company, Spooner, WI), with a 0.2 to 0.4 mm dorsoventral intertip distance. Each of the four threads was soldered onto one of the 5 pins of a miniature female connector; each rat had four stimulation sites: two in the PAG and two in the MH. The connector was then embedded in acrylate resin and anchored to the skull by means of four stainless steel screws, one of which was used as the common anode. Four days after surgery, rats were subjected to an electrode screening test.

Procedures

Electrode Screening Test

This test determined which of the stimulation sites and which intensity would be used for the study. Rats were first observed and screened rapidly for sites that elicited escape behaviors. The screening test was terminated when jumping and explosive behaviors were observed during stimulation of the PAG, or sniffing and rearing behaviors were observed during stimulation of the MH. Each rat was placed in an operant chamber (25 x 25 x 35 cm rectangular box) and allowed to habituate for 10 min. Each of the four sites was stimulated during the same session, in a randomized order, with a two min pause between tests. The stimulation intensity was manually increased (5 or 10 μA steps over 5 sec) to determine the threshold current intensity at which the lowest stimulation

intensity elicited an escape reaction (jump out of the box), with a cutoff values of 70 μA in the discrimination experiment and 220 μA in the switch-off experiment. There was a 15-sec time limit for each step. Threshold intensity for each site was assessed three times, and the three recorded values were averaged. The stimulation was stopped immediately after the observed behavioral response was violent or incompatible with continuing observation, e.g. a violent jump out of the box. In these situations stimulation was interrupted for ethical reasons as well as preventing the rat from injuring itself or dislodging its connector. The electrode site used for both experiments was randomly selected from among those sites that initially produced an escape reaction.

Experiment 1. Discrimination paradigm

Drugs

Diazepam (Sigma, St Louis, MO) was suspended in 3% carboxymethylcellulose; PTZ (Sigma, St Louis, MO) was dissolved in 0.9% saline. Both drugs were prepared fresh daily and given in a volume of 1 ml/kg. Controls consisted of pretreatments with the appropriate vehicle. Pretreatment of vehicle or drug (DZP or PTZ) was given in a randomized order.

Discrimination training

One day after determining the site of stimulation and the intensity of EBS, rats were assigned to either the PAG or MH group based on the electrode screening test. For both groups, the rats were next trained to discriminate between stimulation (S) and no stimulation (NS).

The training EBS (cathodal pulses, duration: 0.1 ms, frequency: 50 Hz) was adjusted in intensity for each rat; individual intensities were between 80% and 100% of the threshold intensity that induced an escape response in the electrode screening test. An escape response is defined as a response to try to jump out of a box. In order to obtain a reinforcer (one 45 mg food pellet), rats were required to press one lever in the presence of stimulation and the other lever in the absence of stimulation. The correct lever assignment for S and NS was counterbalanced between rats. S and NS sessions were applied in a randomized sequence, i.e. NS, S, NS, NS, S, NS, S, S. Rats were then trained twice daily, with training and testing occurring in the same operant box.

Discrimination sessions were as follows: during the first 30 sec, rats received either S or NS; however, the two levers were inactive, i.e. lever press responses were without consequence, and the house-light was off. In the S condition, EBS continued throughout the session. This 30 sec pre-test period was used to insure that rats could perceive the discrimination stimulus (S or NS)

before pressing a lever. At the end of the 30 sec, the house-light was turned on and the levers were activated. Delivery of the first reinforcer was contingent upon emission of the 10th press on the correct lever. Following the first reinforcer, rats had access to further food reinforcement (FR10) for a period of 15 min with a maximum of 50 reinforcers available. At the end of this 15 min period, the house-light was turned off and the levers were inactivated. During a S session, EBS was also terminated at this time. The criterion for a correct session choice was as follows: rats could not emit more than 20 presses on both levers before obtaining the first reinforcer. The criterion for discrimination between the S and NS conditions consisted of 8 out of 10 consecutive sessions with a correct choice. Once animals had successfully met this discrimination criterion, they were entered into the drug testing phase of the study. Test sessions varied from training sessions in three aspects: 1) the lever on which the rat accumulated 10 presses first (selected lever) was the active lever for reinforcement, i.e. there was no correct lever, 2) the total number of reinforcers was limited to four, and 3) the time for each test session was limited to 10 min.

Pharmacological tests

Effects of pentylentetrazole and diazepam on the discriminative properties of EBS of the PAG and the MH. The effects of pretreatment with pentylentetrazole (PTZ 5, 10 or 20 mg/kg; i.p. 10 min presession) were initially assessed against

four stimulation frequencies: 0 (NS condition), 13, 25 and 50 Hz (S condition). The effects of pretreatment with diazepam (2.5 and 5 mg/kg; i.p. 30 min presession) were assessed against three stimulation frequencies: 0, 37.5 and 50 Hz. At this point, the frequencies were readjusted to three frequencies for DZP tests because the EBS lever selection at 0 Hz and 25 Hz were not significantly different. Therefore, the mid-point frequency between 25 and 50 Hz, 37.5 Hz was selected. The control condition consisted of pretreatment with the appropriate vehicle: saline for PTZ and 3% carboxymethylcellulose (CMC) for DZP. Rats were tested at one frequency per test session. Each test session was interspersed with a minimum of two training sessions. The sequence of these two sessions was randomized: i.e. NS/NS or NS/S or S/NS or S/S. Rats were required to make the correct choice in both training session, before the next test session took place. An incorrect choice in one of these training sessions was followed by at least four correctly chosen consecutive S and NS training sessions applied in a randomized order, before being tested again. Rats were pretreated with saline (i.p.) during the training sessions.

Experiment 2. Switch-off paradigm

Drugs

DZP (Sigma, St Louis, MO), ABC (a gift of Schering, Berlin) and FLU (a gift of Hoffman-LaRoche, Basel) were suspended in 3% CMC; PTZ (Sigma, St Louis, MO) and BUS (Sigma, St Louis, MO) were dissolved in 0.9% saline. All

drugs were prepared daily and given in a volume of 1 ml/kg. Controls consisted of pretreatments with the appropriate vehicle. All tests took place in a randomized order.

Procedures

Electrode Screening Test

This screening test was conducted in the same manner as described previously. One day after the electrode screening test, 10 rats were assigned to the PAG group and 5 rats were assigned to the MH group, based on the screening test results.

Switch-off shaping

In the initial phase of this experiment, rats were shaped to approach a lever and ultimately to switch-off the EBS (cathodal pulses, 0.1 ms, 50 Hz, intensity adjusted for each rat) by pressing a lever. Both levers were active, i.e. interrupted the EBS when depressed. During the initial EBS's, the intensity was manually adjusted to produce a mild behavioral activation. When the rat was in the vicinity of a lever, the EBS was manually switched-off by the experimenter. The rat was then progressively reinforced for closer approaches to a lever. During the later part of this shaping phase, the EBS was manually turned off only when the rat made physical contact with a lever. During these shaping sessions, each EBS was limited to 30 sec, and two consecutive EBS's were separated by a 30-sec rest period. The total number of EBS's for each shaping session was

limited to 30. No more than two shaping sessions were conducted per day. Eight PAG rats acquired the switched-off response, and were assigned to the training group. For the switch-off experiment, only the PAG rats were used because their preliminary switch-off response was more stable than that of the MH rats.

Switch-off training

After subjects learned to switch off the EBS by pressing a lever, they were trained once daily for several days, using training sessions that were in all respects similar to shaping sessions. During these training sessions, the intensity of the EBS was slowly adjusted so that the switch-off latency (SOL; in sec) was between 8 and 12 sec at the training frequency of 50 Hz. After rats had acquired a stable baseline (average SOL not varying by more than 20% between three consecutive training sessions), they were tested at four stimulation frequencies: 30, 40, 50 and 70 Hz. All four frequencies were tested in a single session, in a randomized order. Each frequency was tested in a block of 12 EBS yielding 12 SOL's for each frequency. There was a two min rest period between each frequency testing. The intensity was eventually readjusted for each rat so that the ranges of SOL's for frequencies of 30, 40, 50 and 70 Hz were as follows: 25 to 30 sec, 18 to 22, 8 to 12 sec and 4 to 7 sec, respectively. Once stable frequency-response curves were obtained (i.e. less than 20% variation across three training sessions in the average SOL for each frequency),

these frequency-response curves were considered as a baseline curve. The stimulation intensity was then held constant for the rest of the study, and rats were tested with various drug pretreatments: DZP, ABC, FLU, BUS or PTZ.

Pharmacological tests

The effects of DZP, ABC and BUS, PTZ on the SOR were tested. The effects of the BZD antagonist, FLU and its combined effects with DZP or ABC were tested as well.

Anxiolytic drug effects on the SOR

In these tests, a prototypical BZD anxiolytic, DZP, a novel beta-carboline anxiolytic, ABC and a 5-HT_{1A} partial or selective agonist, BUS, were selected. DZP (1.25, 2.5 and 5 mg/kg) and ABC (0.25, 0.5 and 1.0 mg/kg) were dissolved in 3% CMC and injected 30 min prior to each test. After administration of each drug, behavioral effects such as sedation, motor coordination, defecation and urination were observed until the test session was completed.

For the pharmacological test of BUS, single and multiple administration regimens were used since BUS has been reported to have differential effects dependent upon acute vs chronic treatment (Gobbi et al., 1991). For acute administration, BUS (2.5, 5.0 and 10 mg/kg; i.p.) was dissolved in saline and injected 15 min prior to the session. For multiple administration, BUS (2.5 mg/kg) was administered twice (8:00 AM and 8:00 PM) daily for three days. The frequency-response function (SOR) test was then assessed 15 min after the

injection of BUS on the fourth morning. All four frequencies were tested in a single session as described in the "Switch-off training" section above.

Effects of a BZD antagonist alone or in combination with BZD on the SOR

The effects of the BZD antagonist FLU were tested. FLU (0.1, 1.0 and 10 mg/kg) was freshly dissolved in 3% CMC, and administered i.p. 30 min prior to the test session. In addition, combined pretreatments of FLU and DZP or FLU and ABC were assessed and drug pretreatments were as follows: Rats were first pretreated 30 min pre-session with either vehicle (3% CMC) or drug (2.5 mg/kg of DZP or 0.5 mg/kg of ABC). This was followed by a 15-min pre-session pretreatment with either CMC or FLU (0.1, 1.0 or 10 mg/kg). All drug doses were tested individually in a randomized order.

Effects of anxiolytics on the SOR at high frequency of stimulation in addition to regular frequencies

These tests examined whether the effects of the three anxiolytics on the SOR were influenced by motoric disruption. The effects of high doses of DZP (2.5 mg/kg), ABC (0.5 mg/kg), acute BUS (10 mg/kg) and chronic BUS (2.5 mg/kg) on the frequency response function were examined using a high stimulation frequency (100 Hz) in addition to the other four frequencies (30, 40, 50 and 70 Hz).

The effect of PTZ on the switch-off response

PTZ was selected for comparison with the effects of DZP, ABC and BUS on the SOR, since it has been reported to be a prototype anxiogenic drug (Lal and Emmett-Oglesby, 1983). PTZ (5, 10 or 20 mg/kg) was freshly dissolved in saline, and administered 10 min prior to the test session, with each dose being tested in a separate session.

For all pharmacological tests, the frequency response curve was completed in a single session using the four frequencies tested. SOL was recorded and measured as a function of the four frequencies. A single dose of the drug or vehicle was tested per day. Drug treatments were spaced three days apart to minimize carryover effects. During this drug-free period, rats received daily training to maintain stable baseline SOL.

Data analysis

For the discrimination experiment, data were recorded as the percentage of rats choosing the EBS-associated lever at each frequency. The percentage of EBS lever selection after drug pretreatment was compared to the percentage after an appropriate vehicle pretreatment.

For the switch-off experiment, a mean SOL was calculated for each frequency by averaging the last 10 SOL's from each block of 12 SOL's. The first two SOL's for each block of frequencies were discarded because they showed more variability than the remaining 10 SOL's. Mean SOL's for the vehicle

sessions were obtained by averaging SOL's obtained across three vehicle sessions collected for each drug pretreatment. Mean SOL's were subjected to a two-way analysis of variance with repeated measures (Jerrold, 1984), with frequency of EBS and pretreatment as the within subjects factors. Mean SOL's of a high frequency test (100 Hz) were subjected to a t-test to compare with the mean SOL's of control (70 Hz) (Jerrold, 1984).

Histology

Once the behavioral tests were completed, rats were euthanized with a pentobarbital overdose and intracardially perfused with saline followed by 10% formalin. The brains were then extracted and frozen in isopentane (-30 C^0) and cut into 20 μm serial sections using a cryostat microtome. Sections were stained with cresyl violet. The stimulation sites were localized and histologically verified using the corresponding planes of the Paxinos and Watson atlas (Paxinos and Watson, 1986).

CHAPTER III

RESULTS

Histology

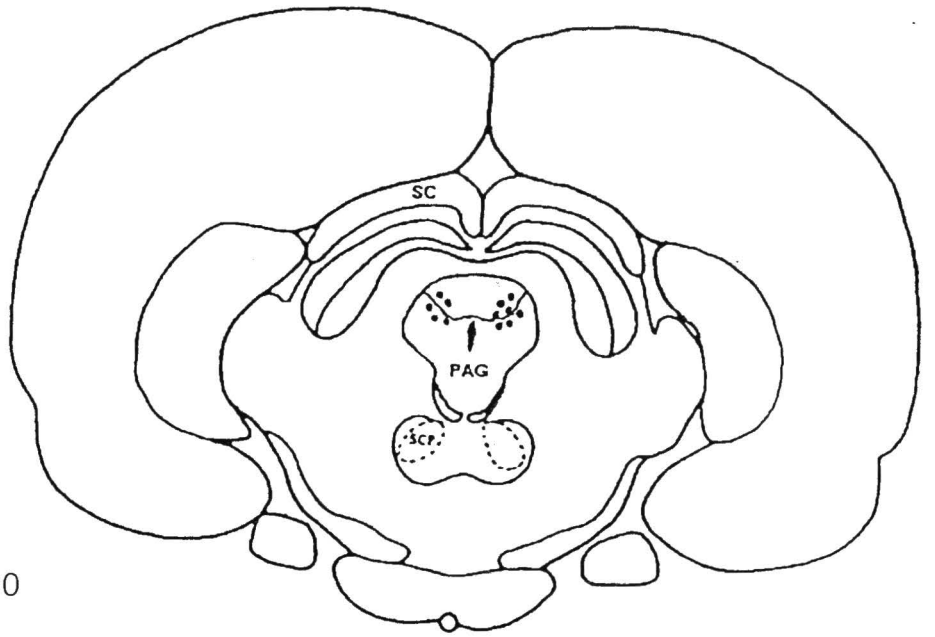
Eleven PAG (Figure 2a) and six MH (Figure 2b) stimulation sites are shown in figure 2. All stimulation sites of rats bearing electrodes aimed for the PAG were located within or dorsal part of the PAG. Electrodes aimed for the MH were located in the medial hypothalamus in the diencephalon. Half of the electrode sites were in the right side and the other half were in the left site of the PAG and MH.

Observed behavioral reaction

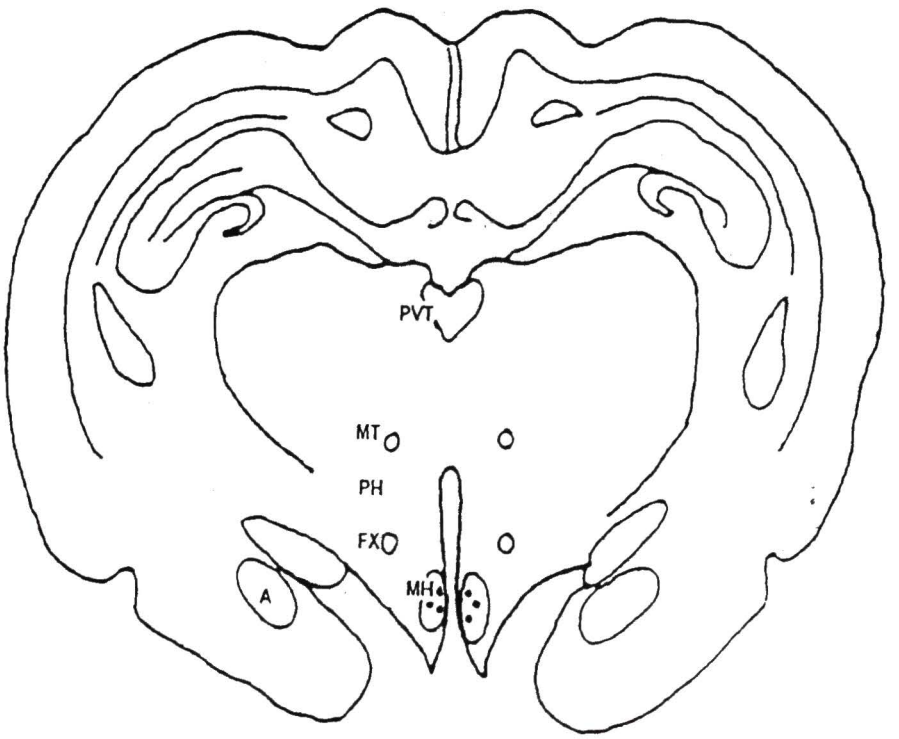
Although stimulation of both structures induced the SOR, differences in the behavior were observed for EBS of the PAG and the MH. When undergoing a PAG stimulation, rats exhibited a freezing posture before manifesting an explosive behavior. The extent of freezing and vocalization was more obvious at higher than at lower stimulation intensities. In contrast, when undergoing a MH stimulation, the rat displays marked locomotor activity including leaps and rearings before jumping in a very coordinated manner to the top of the enclosure wall.

Figure 2. Localization of stimulation sites used in the discrimination paradigm and switch-off paradigm. The electrode tip localizations (i.e. stimulation sites) for the 12 PAG rats and 6 MH rats are shown in Figure 2a and Figure 2b respectively. All stimulation sites of rats bearing electrodes aimed for the PAG were found within the periventricular area of the midbrain, and those for MH in the medial hypothalamus of the diencephalon. Half of the stimulation sites were found in the right side and the other half were in the left side of the brain. The drawings were adapted from the atlas of Paxinos and Watson (1986). Abbreviations: PAG: periaqueductal gray; SC: superior colliculus; SCP: superior cerebellar peduncle. MH: medial hypothalamus; PVT: paraventricular hypothalamus; MT: mammillo-thalamic tract; PH: posterior hypothalamus; Fx: fornix; A: amygdaloid nucleus.

Localization of stimulation sites used in the PAG and the MH rats



Bregma 8.0
Figure 2a



Bregma 4.5
Figure 2b

Experiment 1. Discrimination paradigm

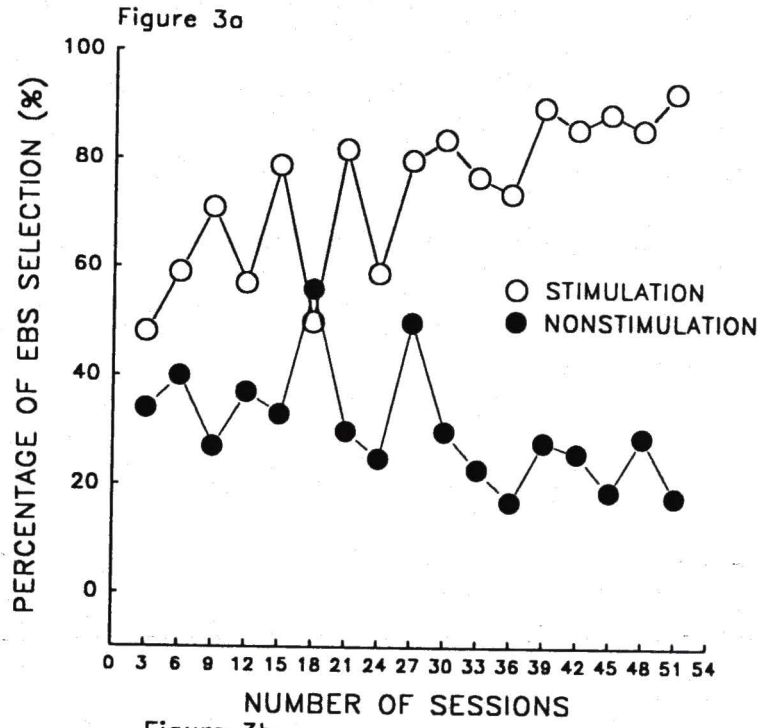
Of the 23 original rats, 21 reached the food shaping criterion and were implanted with stimulating electrodes. Following the electrode screening test, rats were assigned to either the PAG (N=13) or the MH (N=8) group. In the PAG group, during the early stages of discrimination training, one rat lost his head connector, and another rat did not reach the discrimination criterion. In the MH group, two rats did not reach the discrimination criterion. Thus, 11 rats remained in the PAG group and 6 rats in the MH group for pharmacological tests.

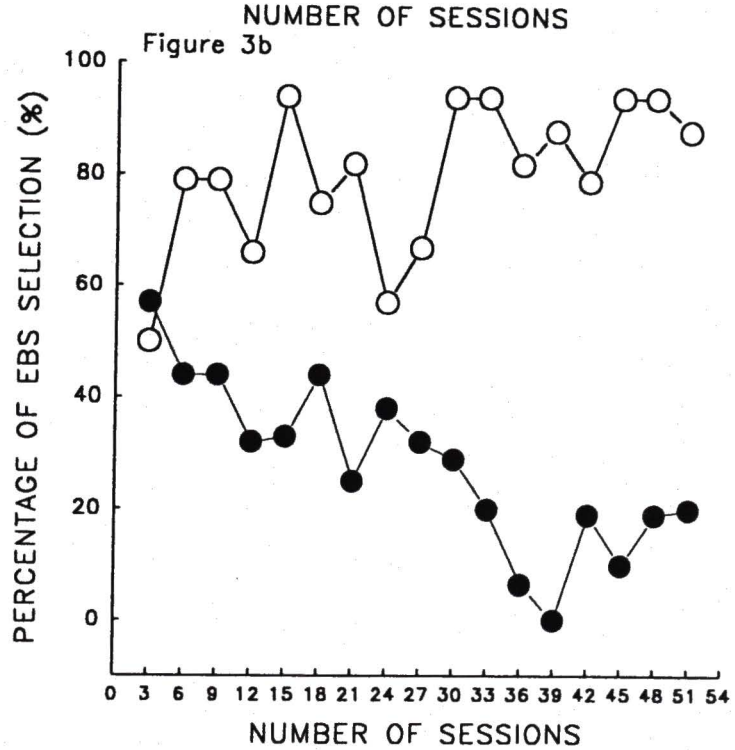
Discriminative responding of rats stimulated in the PAG and the MH

The EBS discrimination tests after vehicle pretreatment were conducted at frequencies of 0 Hz (NS), 13, 25 and 50 Hz. Individual intensities of EBS ranged from 30 μA to 60 μA in both groups. The PAG group had a mean of 51.43 μA (std=14.6, N=11). The MH group had a mean of 43.3 μA (std=0.33, N=6). Figure 3 presents a graphic representation of the discrimination acquisition after EBS of the PAG (Figure 3a) and the MH (Figure 3b). Rats in both the PAG and the MH groups acquired the discriminative responding of the EBS. An asymptote was reached after approximately 45 training sessions in the PAG group and 40 training sessions in the MH group. Throughout the remaining training sessions, the percentage of the EBS selection remained stable (>80 %).

Figures 3a and 3b. Acquisition of the discriminative control of EBS in the PAG and the MH. The discrimination response of rats following EBS in the PAG (Figure 3a) and the MH (Figure 3b) were calculated as the percentage of rats choosing the EBS associated lever in the presence (open circle) or absence (filled circle) of the EBS from the total number of rats responding. Abscissa: number of sessions. Ordinate: percentage of rats selecting the EBS associated lever.

Figure 3. Acquisition of discriminative control of EBS of the PAG and the MH





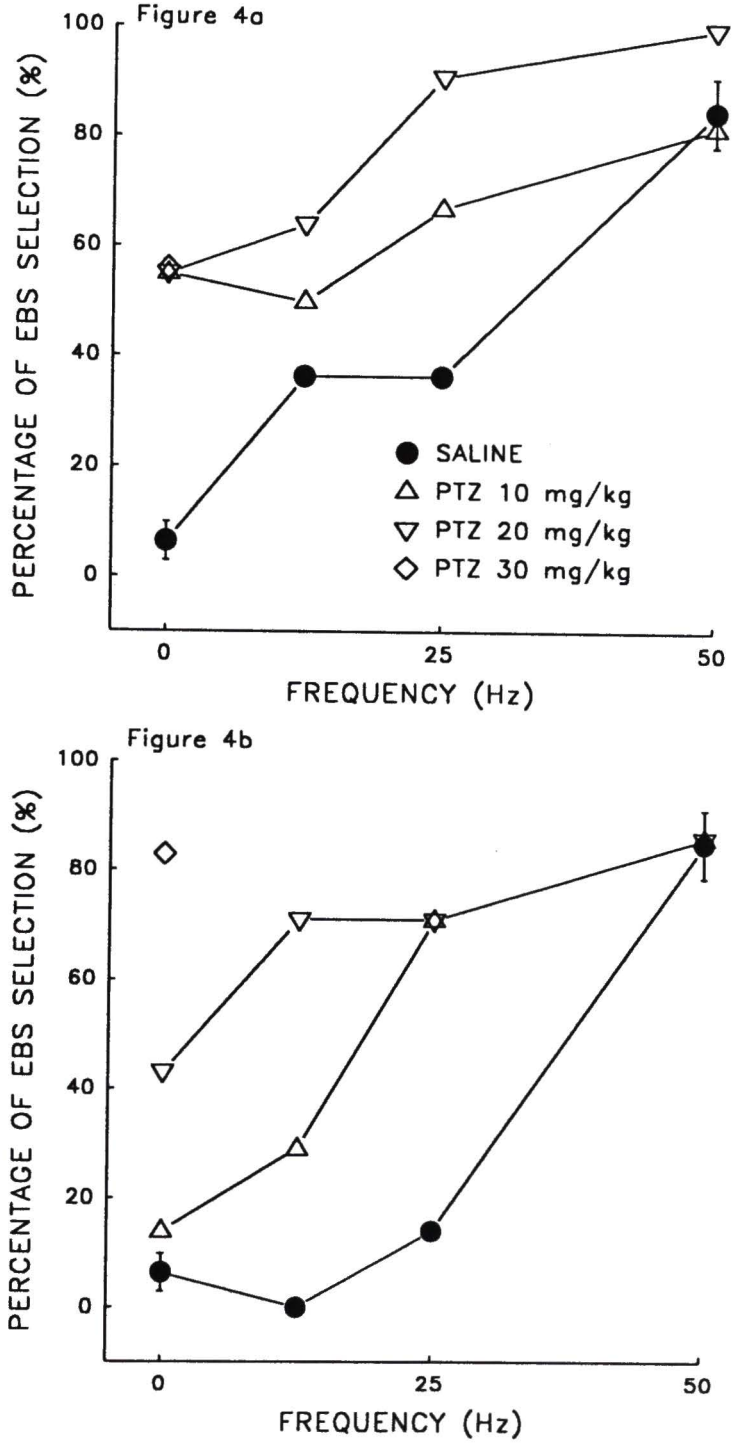
Effects of PTZ on the discriminative stimulus of EBS

Pretreatment with PTZ (10, 20 or 30 mg/kg) dose-dependently increased the number of rats choosing the EBS-associated lever, i.e. potentiated the EBS discriminative stimulus for both groups (PAG, Figure 4a; MH, Figure 4b). The effect of the high dose of PTZ (30 mg/kg) was only tested at 0 Hz in order to avoid the chance of convulsion. The dose-dependent effects of PTZ were more distinguishable at the lower frequencies (0, 12.5 and 25 Hz) than at the highest frequency (50 Hz) for both groups.

To examine the substitution of PTZ for EBS, the percentage of EBS lever selection was reanalyzed at 0 Hz (NS condition) with all doses of PTZ tested (10, 20 and 30 mg/kg). In the PAG group (Figure 5a), the PTZ (0, 10, 20 and 30 mg/kg) substitution for EBS lever selection was 6.2, 55, 55 and 56% respectively. In the MH group (Figure 5b), the corresponding percentages were 6.4, 14, 43 and 83% respectively. The percent of EBS lever selection covaried with the doses of PTZ in the MH group. Since the pretreatment with PTZ (30 mg/kg) in the MH group met substitution criterion (>80%), PTZ (30 mg/kg) substituted for EBS of the MH (83%), and partially substituted (56%), but not in a dose-dependent manner for EBS of the PAG.

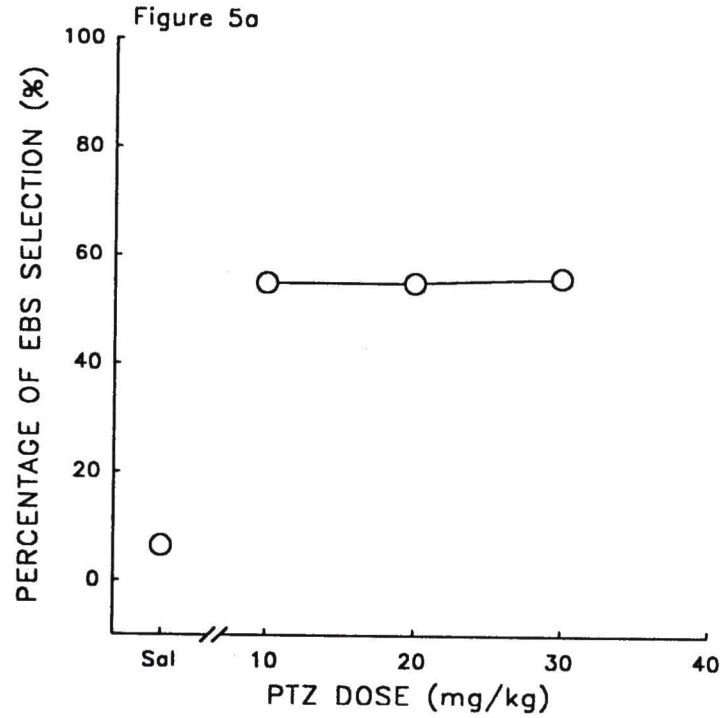
Figures 4a and 4b. Effects of pretreatment with PTZ on discrimination responding for rats stimulated in the PAG (Figure 4a) or the MH (Figure 4b). Drug pretreatments were as follows: saline (filled circle), PTZ 10 mg/kg (open triangle-up), PTZ 20 mg/kg (open triangle-down), or PTZ 30 mg/kg (open diamond). Abscissa: frequency of EBS. Ordinate: percentage of rats selecting the lever associated with the EBS. All symbols represent the mean percent of EBS lever selection. Symbols for 0 and 50 Hz with saline pretreatment represent the average percent of rats choosing the EBS-associated lever during the no-stimulation and stimulation training sessions respectively. These sessions occurred between PTZ test sessions. SEM=3.5 and 8.7 at 0 Hz and 50 Hz respectively in the PAG group. SEM=2.5 and 9.1 at 0 Hz and 50 Hz respectively in the MH group. N=11 (PAG) and N=6 (MH).

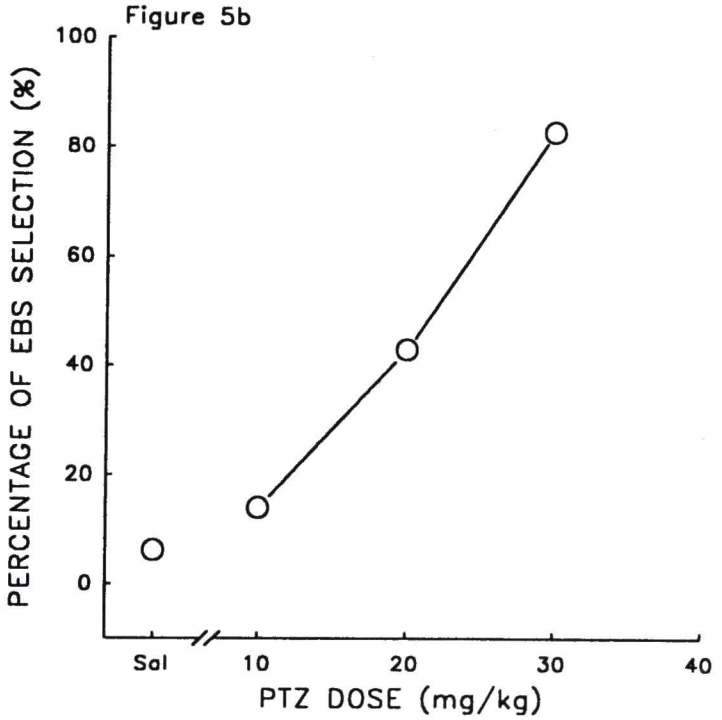
Figure 4. Effects of PTZ on discriminative properties of EBS of the PAG and the MH



Figures 5a and 5b. PTZ substitution for EBS in rats stimulated in the PAG (Figure 5a) or the MH (Figure 5b). Abscissa: PTZ doses in mg/kg. Ordinate: percentage of rats selecting the lever associated with EBS. All symbols represent the mean percent of EBS lever selection after saline (at the dose of PTZ 0 mg/kg) or PTZ (10, 20 and 30 mg/kg) pretreatment. N=11 (PAG) and N=6 (MH).

Figure 5. PTZ substitution for discriminative cue of EBS of the PAG and the MH





Effects of DZP on the discriminative stimulus of EBS

For the PAG group (Figure 6a), pretreatment with DZP (2.5 and 5.0 mg/kg) increased the percentage of rats selecting the stimulation-associated lever at 0 and 37.5 Hz. In contrast, pretreatment with DZP (2.5 and 5.0 mg/kg) decreased the percentage of rats selecting the stimulation-associated lever at 50 Hz.

Likewise, in the MH group (Figure 6b), pretreatment with DZP (2.5 and 5.0 mg/kg) resulted in an increase in the percentage of rats selecting the stimulation-associated lever at 0 Hz, and a decrease in the percentage of rats selecting the stimulation-associated lever at 50 Hz; whereas, at 25 Hz the pretreatment with DZP (5 mg/kg) had no effect. Pretreatment with DZP (2.5 mg/kg) decreased the number of rats selecting the stimulation-associated lever at the frequency of 37.5 Hz.

Experiment 2. Switch-off paradigm

Baseline frequency-response curve of the SOR of EBS of the PAG

The average intensity of the EBS to elicit the stable SOR was 75± 15.9 uA for the 8 subjects. Baseline frequency-response curve (3% CMC) (Figure 7) shows that there was an inverse relationship between the frequency of stimulation and the SOL, so that an increase in the frequency of the EBS resulted in a decrease in the SOL.

Figures 6a and 6b: Effects of pretreatment with DZP on the discriminative response of rats stimulated in the PAG or the MH. For both figures, the pretreatments were as follows: 3% CMC (filled circle), DZP 2.5 mg/kg (open triangle-up) or DZP 5 mg/kg (open triangle-down). Abscissa: frequency of the EBS. Ordinate: percentage of rats selecting the lever associated with EBS. All symbols represent the mean percent of rats selecting the EBS-associated lever. N=11 (PAG) and N=6 (MH).

Figure 6. Effects of DZP on discriminative properties of EBS of the PAG and the MH

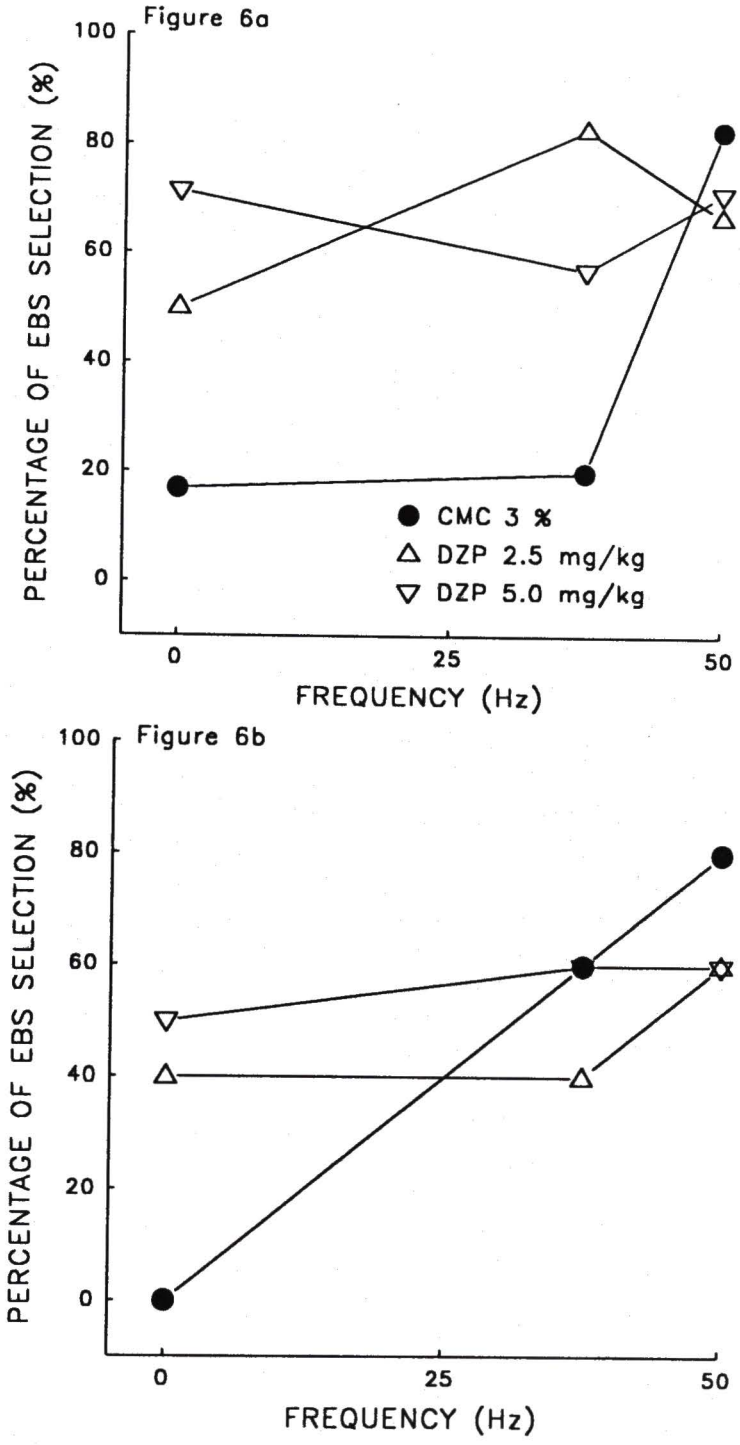
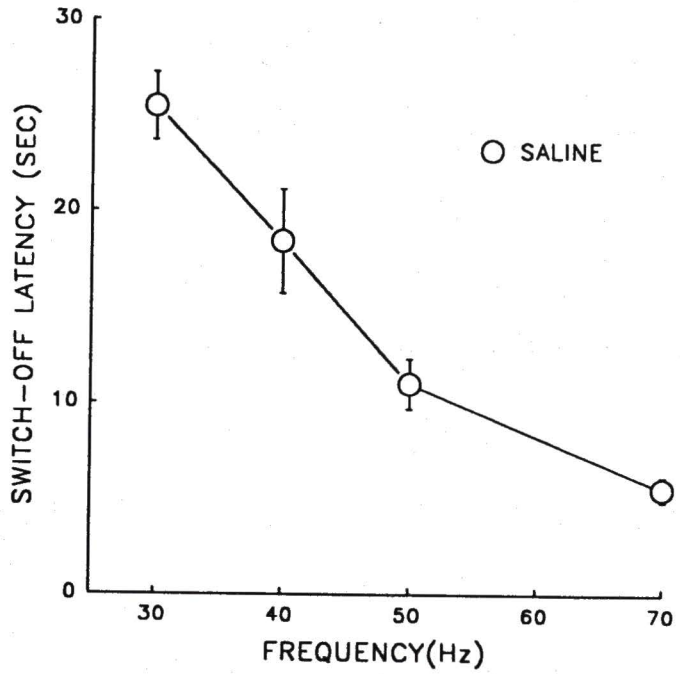


Figure 7. Baseline frequency-response curve of EBS of the PAG



Abscissa: frequency of EBS; Ordinate: switch-off latency (SOL, in sec). Saline was pretreated 10 min prior to the session. All symbols represent the mean SOL and SEM (standard error of mean). N=8. There was an inverse relationship between the frequency of EBS and the SOL.

Effects of DZP and ABC on the switch-off response

There were observed behavioral differences between DZP-pretreated rats and ABC-pretreated rats. DZP pretreated rats appeared weak and sedated after i.p. administration of DZP, especially at the higher doses (2.5 and 5.0 mg/kg) throughout the test session, whereas ABC-pretreated rats did not behave differently than controls. In spite of these observed behavioral differences, DZP (Figure 8a) at doses of 1.25, 2.5 and 5.0 mg/kg and ABC (Figure 8b) at doses of 0.1, 0.5 and 1.0 mg/kg significantly increased the SOL in a dose-related manner across all four frequencies tested [$F(3,113)=6.05$, $p<0.001$; $F(3,84)=8.02$, $p<0.001$ for DZP and ABC respectively, N=8]. The SOL following pretreatment with the low doses of DZP (1.25 mg/kg) and ABC (0.25 mg/kg) did not significantly differ from the SOL after vehicle pretreatment. Interestingly, the degree of increasing effects of DZP and ABC on SOL was nisely ordered by the doses, i.e. the greater the dose is, the greater the increase in the SOL. Note that the doses of ABC that significantly shortened the SOL were five fold smaller than those of DZP.

Effects of BZD antagonist, FLU alone and combined effect of FLU with DZP or ABC on the SOR

Flumazenil (FLU) is a highly selective BZD antagonist in that it acts against CNS effects induced by drugs binding at BZD receptors, but not against those

Figures 8 a,b and c. Effects of pretreatment with DZP (Figure 8a) or ABC (Figure 8b) on the SOR. Drug pretreatments were as follows: 3% CMC (filled circle in both figures). In figure 8a, DZP 1.25 mg/kg (open square), DZP 2.5 mg/kg (open triangle-up) or DZP 5.0 mg/kg (open triangle-down) were pretreated. In figure 8b, ABC 0.25 mg/kg (open square), ABC 0.5 mg/kg (open triangle-up) or ABC 1.0 mg/kg (open triangle-down) were pretreated. Both DZP and ABC significantly increased the SOL in a dose-related manner (Figure 8c) across all four frequencies tested [$F(3,113)=6.05$, $p<0.001$; $F(3,84)=8.02$, $p<0.001$ for DZP and ABC respectively, N=8].

Figure 8. Effects of DZP or ABC on the switch-off latency

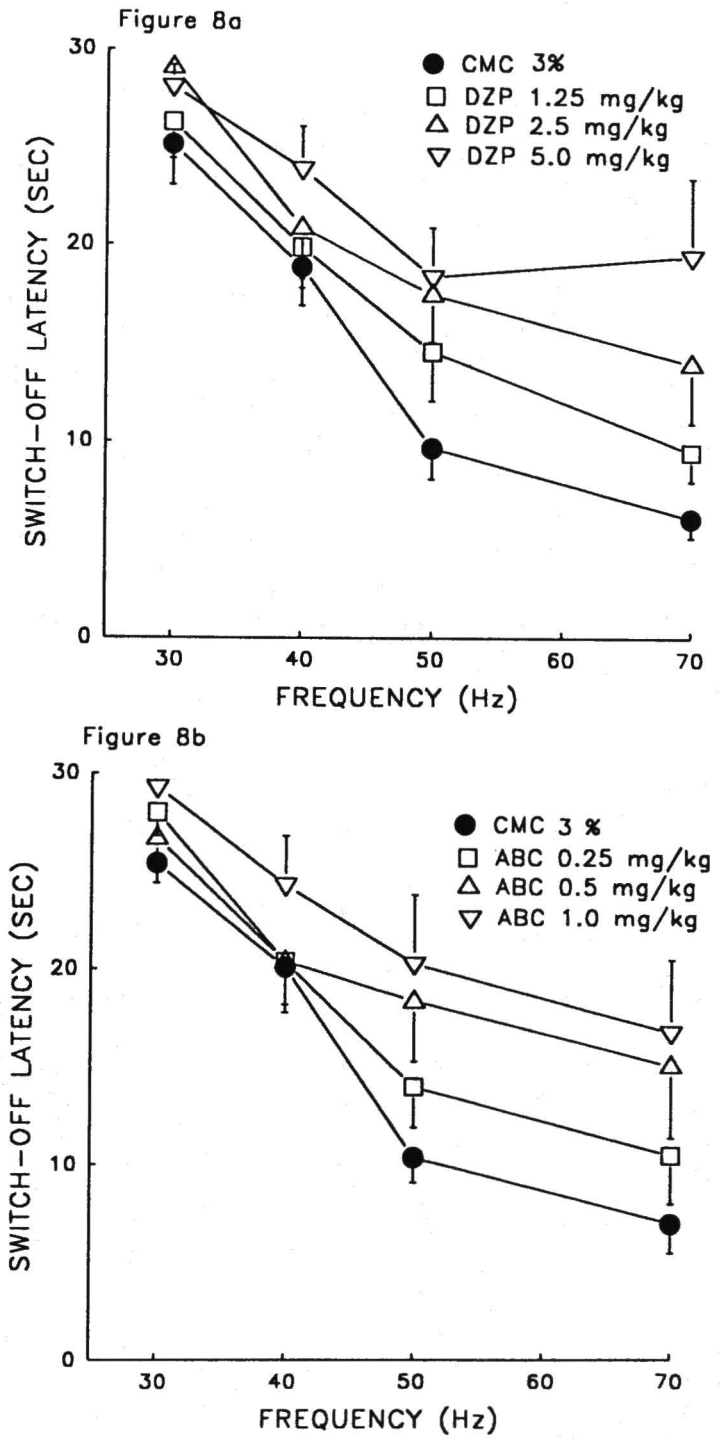
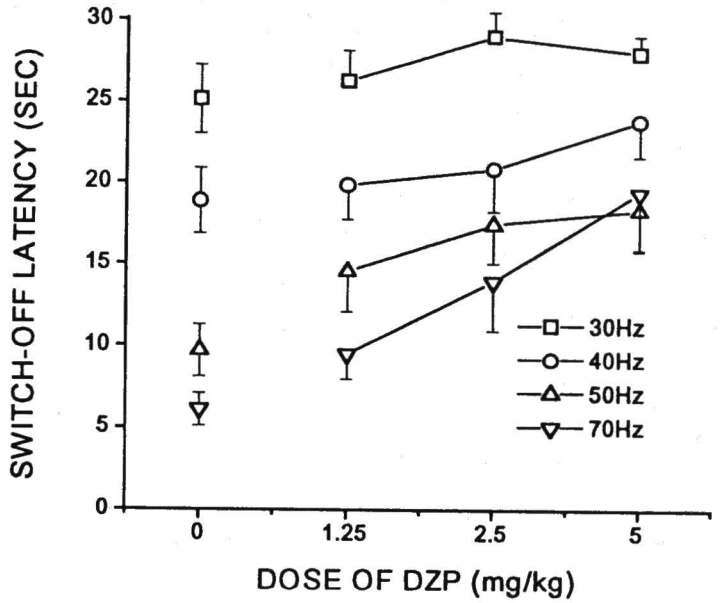
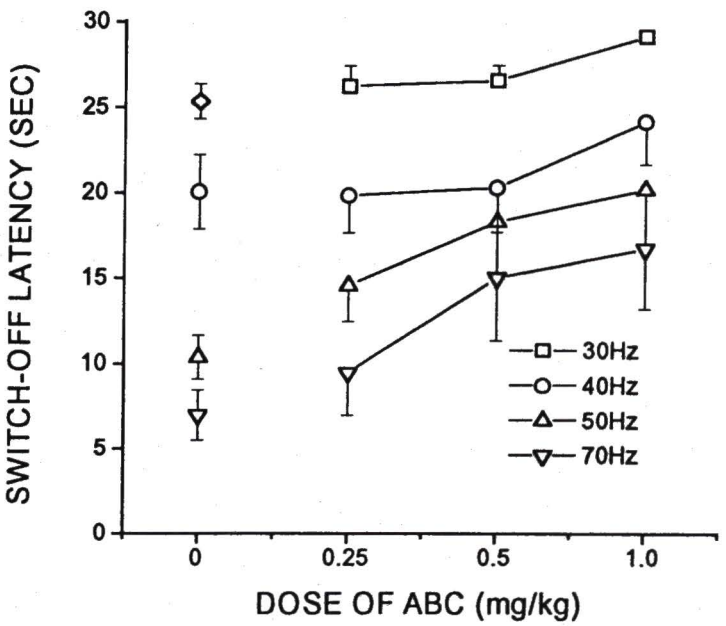


Figure 8c. Dose dependent effects of DZP or ABC on the switch-off latency





produced by other depressants. Figure 9 shows the effects of FLU alone on the
produced by other depressants. Figure 9 shows the effects of FLU alone on the
SOL. Thirty min presession treatment with FLU did not significantly affect the SOL at any dose (0.1, 1.0 and 10 mg/kg) across all four frequencies. However, combined pretreatment with either FLU (10 mg/kg) and DZP (2.5 mg/kg) (Figure 10a) or FLU (10 mg/kg) and ABC (0.5 mg/kg) (Figure 10b) reversed the increased SOL induced by DZP or ABC to the baseline level SOL. Thus, the SOL after combined pretreatment of FLU (10 mg/kg) with DZP or ABC did not significantly differ from the SOL under control condition.

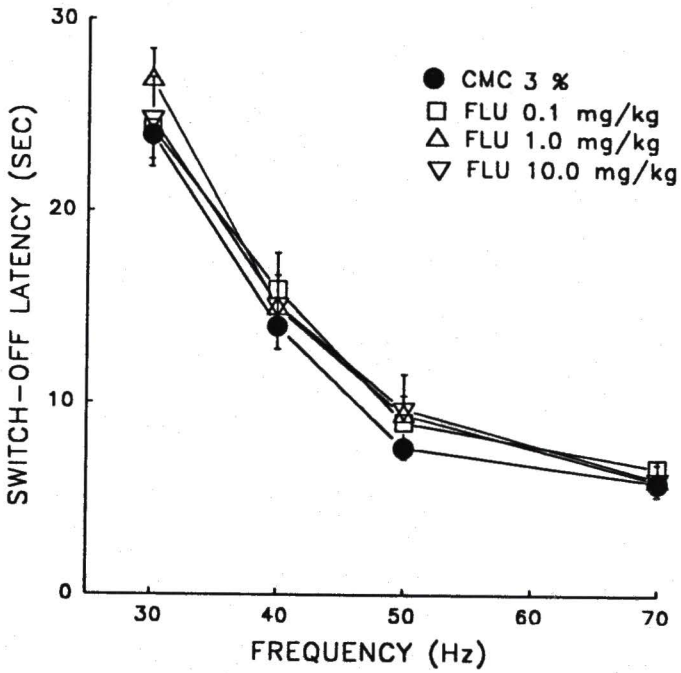
Effects of DZP and ABC on the SOR at high frequency in addition to regular frequencies

The effects of high dose of DZP (2.5 mg/kg) or ABC (0.5 mg/kg) on the SOL of the PAG at high frequency of 100 Hz are shown in figures 11a and 11b. The SOL after pretreatment with high dose of DZP 2.5 mg/kg (Figure 11a) or ABC 0.5 mg/kg (Figure 11b) at 100 Hz did not significantly differ from the SOL under control condition at 70 Hz. Thus, the increased SOL induced by the high dose of DZP or ABC was shortened to the baseline level of 70 Hz by increasing frequency up to 100 Hz.

Effects of acute and chronic administration of anxiolytic buspirone on the SOR

The effects of acutely administered BUS (1.25, 2.5, 5.0 and 10 mg/kg) are shown in figure 12. The pretreatments with BUS at all three doses (1.25, 2.5

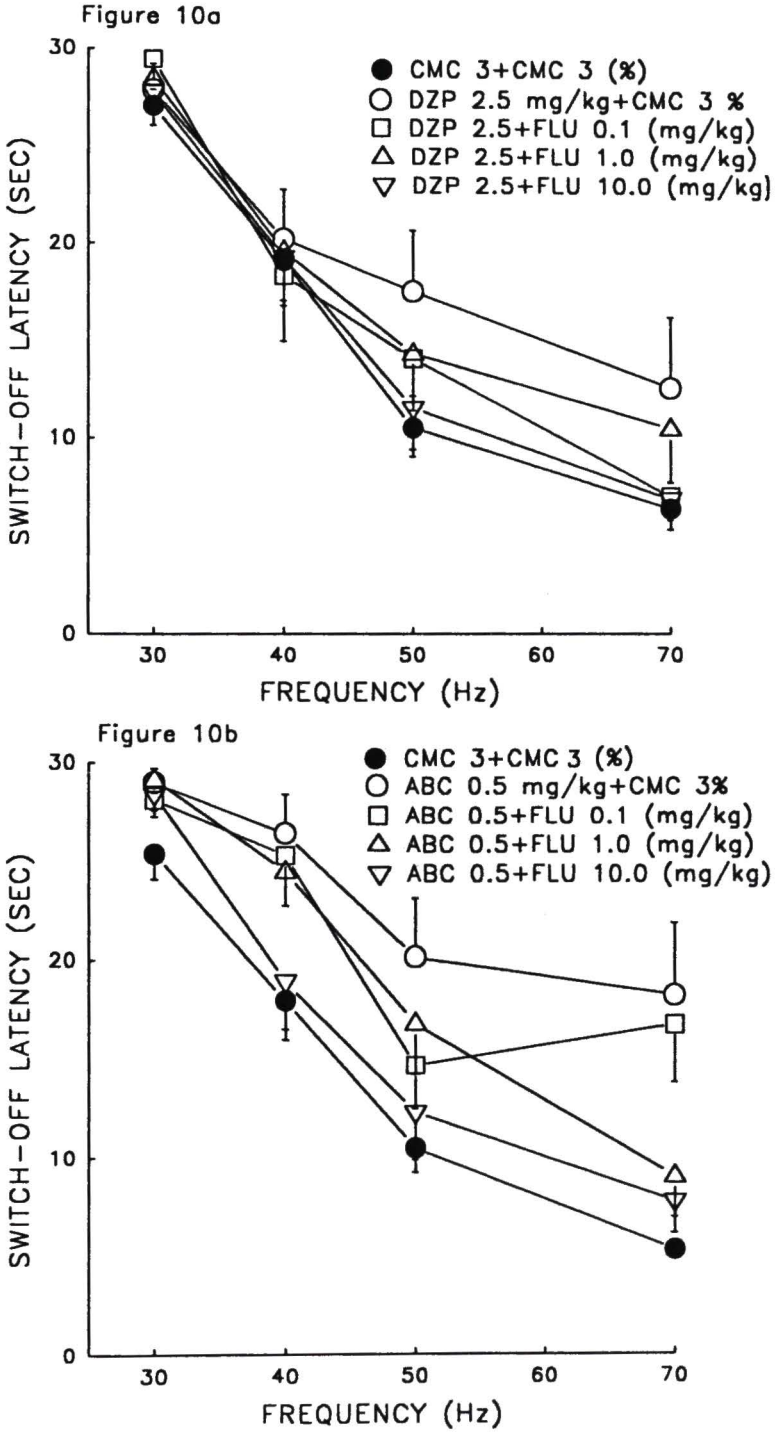
Figure 9. Effects of FLU alone on the switch-off latency



Thirty min presession pretreatment with FLU alone, at all doses tested, did not significantly modify the SOL across all four frequencies tested. N=8.

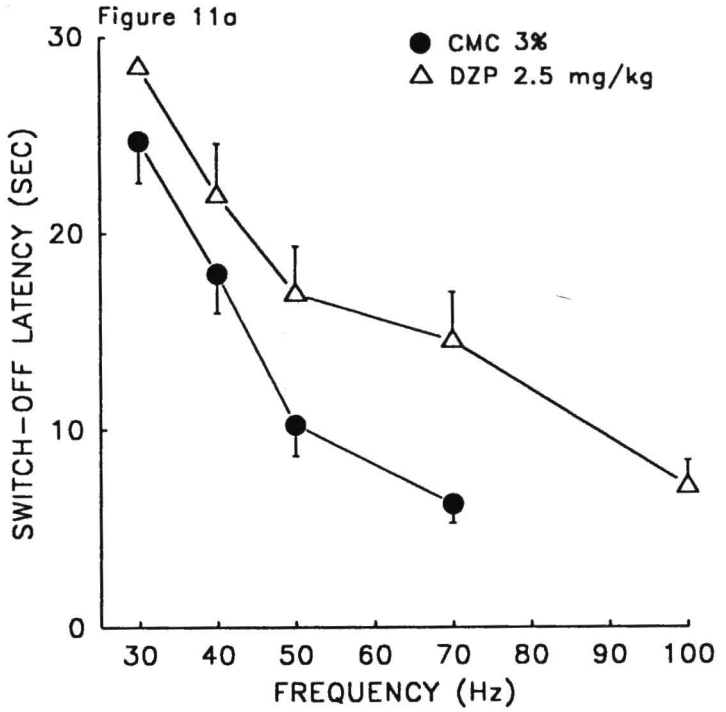
Figures 10a and 10b. Combined effects of FLU with DZP, 2.5 mg/kg (Figure 10a) or ABC, 0.5 mg/kg (Figure 10b) on the SOR. For both figures, combined drug pretreatments were as follows: the first drug was given 30 min prior to the session, the second drug was given 15 min presession: 3% CMC followed by CMC (filled circle), DZP or ABC followed by 3% CMC (open circle), DZP or ABC followed by FLU 0.1 mg/kg (open square), DZP or ABC followed by FLU 1.0 mg/kg (open triangle-up), DZP or ABC followed by FLU 10.0 mg/kg (open triangle-down). The SOL after combined pretreatment with either FLU, 10 mg/kg and DZP or ABC did not significantly differ from the SOL under control condition (CMC and CMC combined pretreatment).

Figure 10. Combined effects of FLU with DZP or ABC on the switch-off latency



Figures 11a and 11b. Effects of DZP and ABC on the SOR at high frequency in addition to regular frequencies. Following 30 min presession pretreatment, the effects of DZP, 2.5 mg/kg (open triangle-up, Figure 11a) or ABC 0.5 mg/kg (open triangle-up, Figure 11b) were tested at five frequencies, 30, 40, 50, 70 and 100 Hz in a randomized order. SOL after pretreatment with DZP or ABC at high frequency of 100 Hz did not significantly differ from the SOL under control condition at frequency of 70 Hz.

Figure 11. Effects of DZP or ABC on the switch-off latency at a high frequency additional to the regular frequencies.



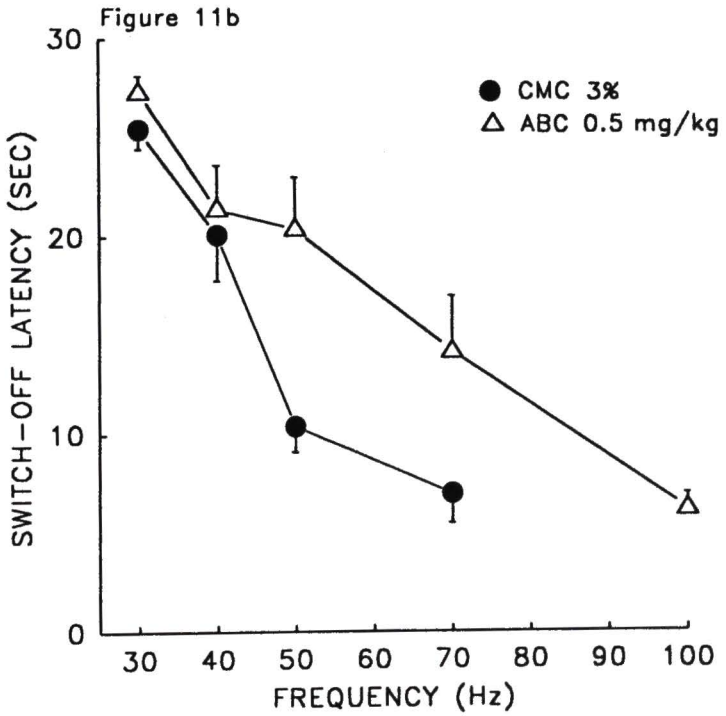
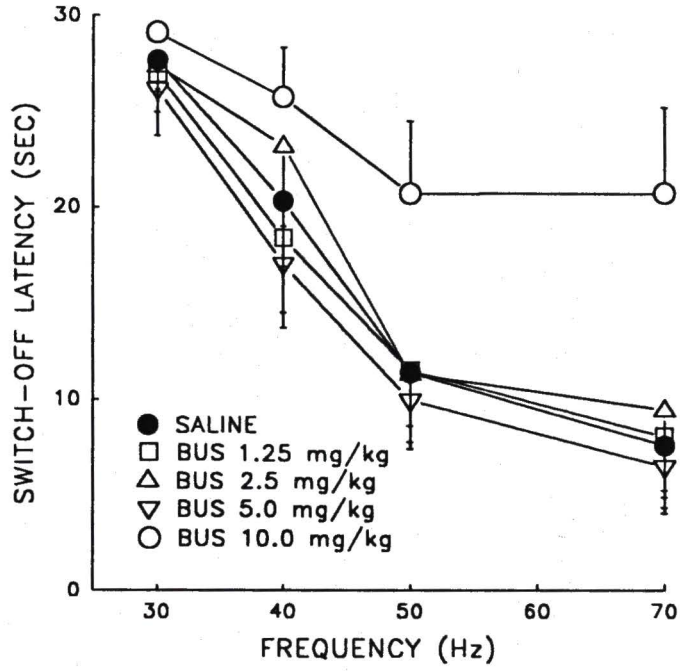


Figure 12. Effects of acute BUS on the switch-off latency



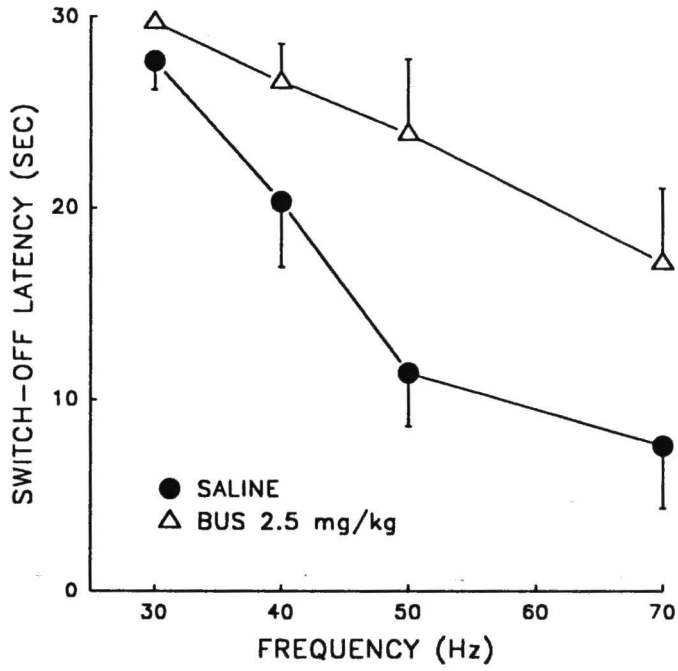
BUS was pretreated 15 min prior to the session. SOL after pretreatments with low doses of acute BUS (1.25, 2.5 and 5.0 mg/kg) did not significantly differ from the SOL of control (saline). In contrast, pretreatment with a high dose of acute BUS (10 mg/kg) significantly increased the SOL [$F(1,16) = 26.553$, $p < 0.0001$]

and 5.0 mg/kg) did not significantly modify the SOL. In contrast, the SOL following pretreatment with acute BUS (10 mg/kg) was significantly increased [$F(1,16)=26.553$, $p< 0.0001$, N=6]. Acute administration of BUS (5.0 mg/kg) showed a tendency to shorten the SOL, though this trend was not statistically significant. Figure 13 shows the effects of chronic BUS on the SOL. On the fourth morning following the last injection of the multiple regimen of BUS (2.5 mg/kg, twice/day for three day), 15 min presession pretreatment with BUS 2.5 mg/kg significantly increased the SOL across all four frequencies (Figure 13) [$F(1,20)=19.036$, $p<0.0001$, N=6].

The effects of BUS on the SOR at a high frequency in addition to regular frequencies

The effects of acute BUS (10 mg/kg) and chronic BUS (2.5 mg/kg) on the SOL were assessed at high frequency of 100Hz to examine whether the effects of BUS on the SOL are influenced by motoric consequence. The increased SOL induced by acute BUS (10.0 mg/kg) (Figure 14a) was not shortened whereas the increased SOL induced by multiple regimen of BUS (2.5 mg/kg) (Figure 14b) was reversed to the baseline level of 70 Hz by increasing the frequency to 100 Hz. Thus, SOL after acute BUS (10 mg/kg) at all frequencies tested remained significantly higher than SOL of 70 Hz after vehicle treatment [$t(10) = -3.235$, $p < 0.001$].

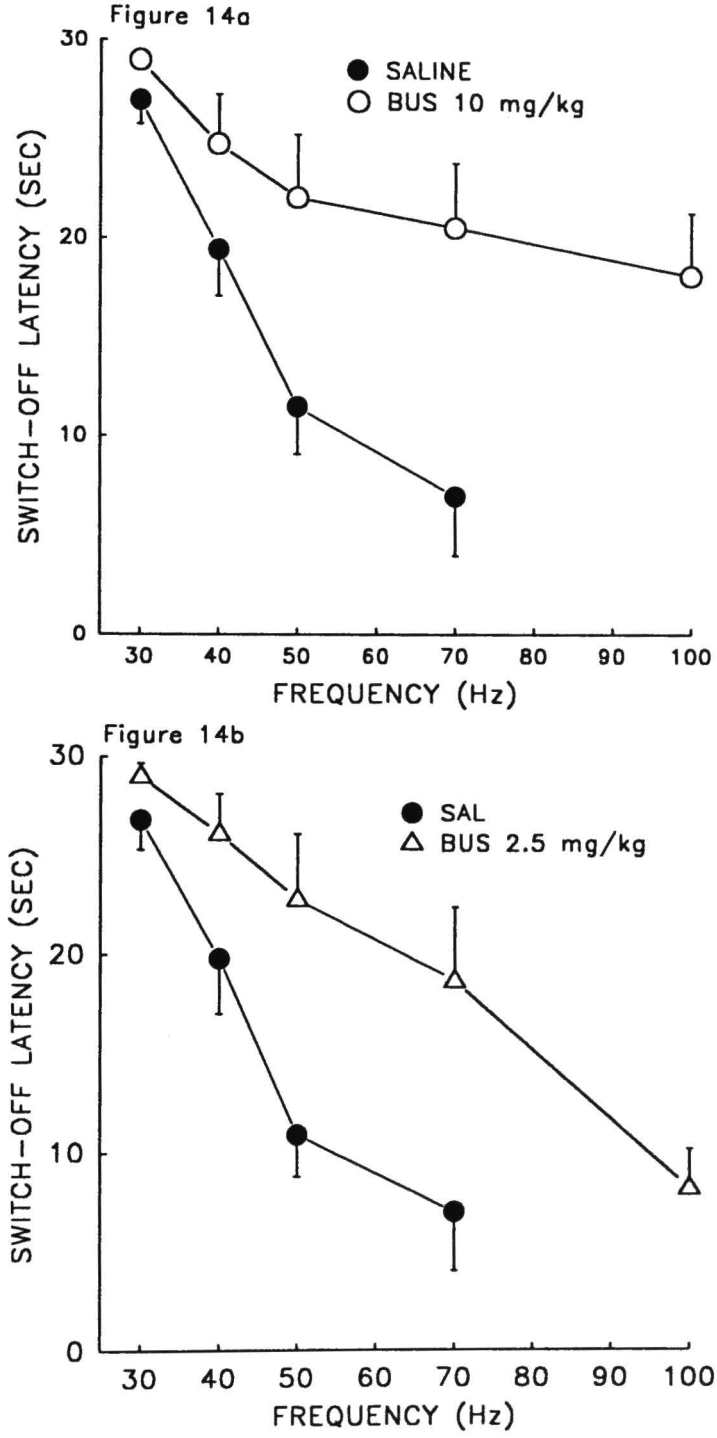
Figure 13. Effects of chronic BUS on the switch-off latency



Chronic BUS (2.5 mg/kg) was administered twice a day for three days. On following fourth morning, 15 min presession pretreatment with BUS (2.5 mg/kg) significantly increased the SOL [$F(1,20) = 19.036$, $p < 0.0001$, $N=6$].

Figures 14a and 14b. Effects of acute (Figures 14a) and chronic (Figures 14b) BUS on the SOR at high frequency of 100 Hz. The increased SOL by acute BUS 10 mg/kg (open circle) was not shortened by high frequency of 100 Hz. Thus, SOL of 100 Hz after acute BUS (10 mg/kg) was significantly greater than SOL of 70 Hz after control [$t(10)= -3.235$, $p<0.001$, N=6]. In contrast, the increased SOL by chronic BUS (2.5 mg/kg, open triangle-up) was reversed to the baseline level (filled circle) of 70 Hz by increasing frequency up to 100 Hz.

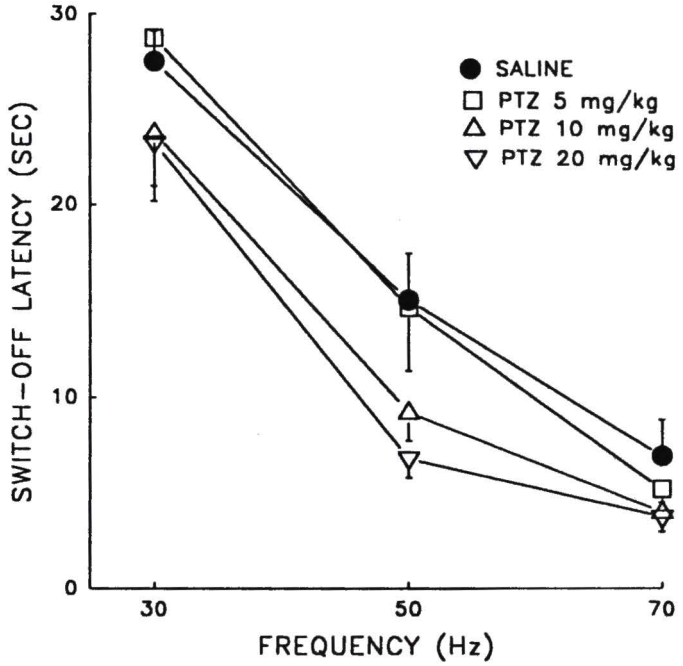
Figure 14. Effects of acute and chronic BUS on the switch-off latency at a high frequency additional to the regular frequencies.



Effects of PTZ on the SOR

The effects of PTZ on the SOR of rats stimulated in the PAG are shown in figure 15. Ten min presession pretreatment with PTZ (5, 10 and 20 mg/kg) significantly shortened the SOL of the EBS at the PAG in a dose-dependent manner across all three frequencies tested [$F(3,15)=5.16$, $p<0.01$, N=6]. Pretreatment with the lower dose of PTZ (5 mg/kg) did not significantly modify the SOL, as compared to the SOL after vehicle treatment.

Figure 15. Effects of PTZ on the switch-off latency



Ten min presession pretreatment with PTZ significantly decreased the SOL in a dose dependent manner [$F(3,15) = 5.16$, $p < 0.01$, $N=6$.]

CHAPTER IV

DISCUSSION

The behavioral differences between the PAG and the MH stimulation

Although EBS of either the PAG or MH elicit a SOR, there are differences in the behaviors induced by the stimulation of these brain areas. As observed in this study, PAG-stimulated rats show a defensive posture manifested by an immobile, crouched display and escape behavior (passive escape) whereas MH-stimulated rats exhibit marked locomotion interspersed with leaps and grooming (active escape). These behaviors are also shown by rats under threatening or defensive situations (Kiser and Lebovitz, 1975; Olds and olds, 1962), indicating that these areas are part of an integral fight-flight system (Gray, 1975). These two types of escape behaviors (Robinson, 1978; Waldbillig, 1975) are unlikely to be due to the mere stimulation of two opposing motor patterns, but may represent two potential responses to an underlying NRS generated by the EBS. Preventing the rat from switching-off the stimulation by blocking the lever results in the reappearance of the escape behavior observed in the present study. These escape behaviors disappear during acquisition of the SOR, i.e. the animal learns to substitute lever pressing for the original response.

If one assumes that the particular overt escape behavior reflects an underlying NRS effect, it follows that the nature of the two NRS differ between the PAG and the MH stimulation. If so, this might be due in part to the fact that the stimuli induced by PAG or MH stimulation are not due to the activation of a single fiber system. This hypothesis is supported by the following evidence: 1) a PAG lesion was found to attenuate, but not abolish, SOR induced by MH stimulation (Schimitt, et al., 1979). 2) a unilateral MH lesion attenuates the SOR induced by ipsilateral PAG stimulation, while the contralateral SOR induced by stimulation at PAG sites to the MH lesion were slightly or not at all affected (Sandner et al., 1987). 3) Since some fibers of the MH pass through the lateral hypothalamus, MH stimulation is found to produce both negative and positive reinforcing effects (Schimitt et al., 1979). While the dorsal PAG stimulation induced only negative reinforcing effects (Schimitt et al., 1979). So far no systematic studies have been done to establish the exact neuronal systems to account for the different stimulus effects induced by PAG and MH stimulation.

Discrimination paradigm

A two-lever food-reinforced operant task, performed by rats in Skinner boxes, is well suited to test stimulus discrimination, because it yields easily quantifiable data about the detectability of the stimulus as compared to

other behavioral procedures. In addition, rats emit the same operant response under a wide variety of treatment conditions.

The results from the two-lever food-reinforced discrimination paradigm obtained in this study demonstrate that rats discriminate the presence or the absence of brain stimulation of the PAG and the MH. Figures 3a and 3b present a graphic representation of discrimination performances, demonstrating the appropriateness of the animals' response to the two conditions defined as the presence or absence of brain stimulation. The average number of sessions needed to acquire a discriminative stimulus varies between laboratories. This is because the operational criteria for discrimination vary to some extent. In the present work, the majority of rats reached the discrimination criterion in an average of 54 training sessions. Similarly, the number of sessions required to reach an equivalent criterion for discrimination of EBS in the mesencephalic locomotor region was 59 (Depoortere et al., 1990c).

The discriminative stimulus properties of EBS of the dorsal PAG or the MH have been investigated in previous studies (Jenck et al., 1986; Lappuke et al., 1982, 1988). In those studies, the combination of the SOR and T-maze procedure was used; a rat switch-off one lever located in one arm of T-maze and another lever located in another arm, when stimulated in the PAG and the MH, respectively.

The differencies between the paradigm in the present study and those studies are as follows: 1) In a two-lever food-reinforced discrimination paradigm, a rat discriminates between stimulation and no stimulation. In that study, a rat discriminates between two different EBS, i.e. EBS applied to two different brain sites (the PAG and the MH) or EBS with two different stimulation intensities. 2) A motivating stimulus is a food reinforcer in the present study vs a negative reinforcing brain stimulation. 3) The present paradigm only requires low intensity brain stimulation (30-60 μA) to induce a rat to press a lever whereas high intensities of the EBS (40-250 μA) are required to switch-off the lever. Consistently, the current intensities (50-220 μA) used in the present switch-off paradigm were higher than those used in the discrimination paradigm.

The advantage of a two-lever food-reinforced discrimination paradigm could be such that it dissociates in some degree, the discriminative stimulus from the negative reinforcing stimuli. Using a same paradigm, Depoortere et al. (1990c) demonstrated that a rat discriminated the presence and the absence of MLR stimulation.

Figures 4a and 4b show pharmacological tests conducted with a prototypic anxiogenic drug, PTZ (Corda et al., 1983; Dorow et al., 1983; File et al., 1985). Under control conditions, the percentages of EBS-lever selection at 0, 13.5, 25 and 50 Hz were 8.6, 38, 38 and 83 % and 8.3, 0,

12.5 and 82 % in the rats stimulated in the PAG and the MH sites, respectively. These results indicate that the probability to discriminate EBS of the PAG and the MH increases as the strenghth of the stimulation increases, i.e. the higher the frequency, the higher the percentage of EBS-lever selection. Thus, it appears that the discriminative stimulus effect is likely to increase when the strenghth of the PAG and the MH stimulation increases. This is consistent with the result from other negatively reinforcing brain structure, MLR (Druhan et al., 1987; Hayes and Mayer, 1978; Depoortere et al., 1990a). There was a covariation between the EBS-lever choice and the strength of the stimulation.

A similar phenomenon was shown in a discrimination study of the positive reinforcement system; the discriminative cue induced by positive reinforcing drugs, cocaine or LSD substituted for the discriminative cue induced by positive reinforcing EBS of the lateral hypothalamus (Colpaert, 1977a).

The results with PTZ pretreatments show that the percentage of EBS-lever selection increased following the PTZ administration in a dose dependent manner. These effects were more distinct at low frequencies (0, 12.5 and 25 Hz) than at high frequency (50 Hz). Since the percentage of rats selecting EBS-lever was already high (>80 %) at frequency of 50 Hz under control condition, further increase of this percentage might have

been difficult. In any case, it appears that PTZ-induces potentiation of the discriminative cue produced by the PAG and MH stimulation.

Considering the PTZ substitution data (Figures 5a and 5b), the EBS-lever selection under no stimulation condition (0 Hz) reached 55 %, 55 % and 56 % after PTZ pretreatment at the doses of 10, 20 and 30 mg/kg, respectively in the PAG group. In the MH group, the corresponding numbers were 13, 43 and 83 %, indicating that PTZ dose-dependently substituted for the discriminative cue of MH. Thus, at the dose of 30 mg/kg, PTZ partially substituted (56 %) and fully substituted (83 %) for the PAG and the MH stimulation, respectively.

Complete substitution between EBS and systemic drug administration is infrequent. EBS, although activating only a restricted amount of neural tissue, may be presumed to produce a number of stimulus effects, some, or all of which may gain stimulus control over the behavior of any given subject. Systemic drug administration would affect an even greater neural substrate and should be expected, therefore, to produce an even greater number of potential cues. This multiplicity of cues theoretically reduces the probability that the same cues will gain stimulus control over the behavior of a group of subjects in both conditions. In addition, the different neuronal systems innervating two structures may account for the different degrees of substitution.

The PTZ data demonstrated that pretreatment with PTZ not only potentiated (in the PAG and the MH group) the discriminative stimulus but also substituted for that stimulus (in the MH group). Thus, it may be assumed that the brain stimulation of the PAG and the MH are, in some degree, interchangeable with the effects of PTZ on discriminative responding in this paradigm. Because the discriminative stimulus associated with the injection of PTZ (Shearman and Lal, 1979) has been proposed as a model of anxiety (Lal and Emmett-Oglesby, 1983), it can be speculated that the discriminative stimulus effects induced by EBS of these brain areas can also act as an anxiogenic stimulus in this anmal model.

Regarding the results with DZP (Figures 6a and 6b), pretreatment with DZP increased the EBS-lever selection at low frequencies whereas it tended to decrease it at frequency of 50 Hz in both groups. Since an anxiogenic, PTZ, potentiated the discriminative cue, DZP was expected to block that cue. However, present results appear that the cue produced by DZP substitutes for discriminative cue of EBS when animals perceive no stimulation or low stimulation. On the other hand, when animals perceive distinct stimulation, DZP appears to attenuate discriminative cue of EBS. If so, the failure of DZP to block discriminative cue of EBS can be interpreted as being due to the fact that the stimulation parameter used in the discrimination task may not have had as strong a stimulus effect as

predicted. In fact, the adjusted intensities for each rats to acquire the discriminative responding were in the range of 30 to 60 μA, which is lower than that used in switch-off experiment (50 to 210 μA) in this study. The frequencies tested were 0 to 50 Hz vs 30 to 70 Hz in discrimination paradigm and switch-off paradigm, respectively. This observation gives us two points of view: 1) A high stimulation is not necessary for animals to discriminate the stimulation. 2) It requires a high stimulation in order to act as a distinct NRS to detect anxiolytic drugs.

A similar phenomenon was seen in a FLU discrimination study (Rowan and Lucki, 1992). Rats were trained to discriminate the stimulus effects of FLU using a conditioned taste aversion procedure. On saline trials, rats injected with saline consumed a normal amount of saccharin solution. On drug trials, rats injected with FLU (32 mg/kg) paired with LiCl decreased saccharin consumption. Acquisition of the discriminated taste aversion, as measured by differential effects on saccharin consumption between saline and drug trials, developed after five pairings of FLU with LiCl injections. In substitution tests, rats trained with low doses of FLU (1mg/kg) substituted for both BZD receptor antagonists (CGS 8216 and ZK 93426) and a BZD agonist (CDP), while rats trained with high (32 mg/kg) doses of FLU substituted only for BZD receptor antagonists. By analogy, it could be

hypothesized that the stimulation parameters used to train the animals were detectable, but not adequate to produce a reliable discrimination.

Thus, it needs to be determined using a higher intensity stimulation parameter for training whether DZP would produce the predicted result. At the very least, it can be useful to provide the discriminative cue between stimulation and no stimulation of the PAG or the MH.

Base-line frequency-response curve of the SOR

There was an inverse relationship between the EBS frequency (1/interpulse interval) of the PAG stimulation and the SOL (Figure 7). Thus, under baseline conditions, a greater strength of the stimulation resulted in a shorter SOL. This inverse relationship was also observed in rats stimulated in the MLR (Depoortere et al., 1990a). Since the PAG and the MLR are implicated in the fight-flight system (Amano et al., 1978), the decrease in the latencies by increasing the frequency (Figure 7) is likely to result from an increase in the NRS of the EBS.

It is a widely held opinion that a change in stimulation intensity results in a change in the number of directly activated neurons (spatial summation), while a change in stimulation frequency alters the firing rate of an unchanged number of directly activated neurons (temporal summation) (Ranck, 1975). Thus, when higher intensities are used, the volume of

exited neuronal tissue is assumed to increase (Ranck, 1975). This may reduce the site specificity of EBS applied to the target brain area. Indeed, Depoortere et al. (1990a) pointed out this aspect in SOR of rats stimulated in the MLR. The SOL was measured by varying the interpulse interval under two conditions: one group of rats were stimulated with fixed high intensity, the others were with fixed low intensity. Under each condition, when the interpulse interval was reduced (when the frequency was increased), the effect of changing the interpulse interval on SOL was less effective in the high intensity group than in the low intensity group. The authors suggested that the stimulation with high intensity was less effective in decreasing the SOL than low intensity. This phenomenon was interpreted as being due to the following fact. The volume of exited neuronal tissue is increased followed by the limiting effect of the increased activation of local inhibitory interneurons and/or the increased activation of redundant neural circuitry. Therefore, in order to avoid the non-specific stimulation in the target site by changing current intensity, changes in frequency were used as a variable parameter in the present study.

Effects of DZP and ABC on the SOR

The results shown in figures 8a and 8b demonstrate that the SOL is dose-dependently increased by DZP (1.25, 2.5 and 5 mg/kg) and ABC (0.1,

0.5 and 1.0 mg/kg). These results were reanalyzed as a function of dose to illustrate dose-dependent effects of DZP or ABC on the SOL (Figure 8c). Thus, dose-dependently increasing effects of these drugs were more distinctive at high frequency stimulation (50 and 70 Hz) than at low frequencies (30 and 40 Hz). These effects are unlikely to be due to a non-specific behavioral depression. If the animal's motoric capacity were disturbed by these drugs, a rat would not have performed the SOR as fast at high frequency (100 Hz). However, the increased SOL induced by these drugs were reversed to the baseline level when the frequency was increased to 100 Hz (Figures 11a and 11b). Thus, the effects of DZP and ABC on SOR are likely to result from the attenuation of the NRS rather than from a general motoric suppression.

The effect of DZP on the NRS was previously shown by Bovier et al. (1982). In their study, rats stimulated at the PAG were trained to escape from one compartment of a shuttle box to another, consequently turning off the EBS. The escape latency (in sec) and the number of spontaneous passages were recorded as indices of the stimulus effect and the motoric capacity, respectively. DZP (5 and 7.5 mg/kg i.p.) increased escape latency, but did not decrease the number of spontaneous passages. In addition, DZP blocked other signs of distress such as vocalizations, piloerection and jumping.

Using a switch-off paradigm, Schenberg and Graeff (1978) investigated the effects of CDP on SOR induced by PAG stimulation. The switch-off paradigm in that study is slightly different from the one used in the present study. They adjusted the stimulus intensity for each animal in order to produce a SOL in the range of 4 to 6 sec per session. Drug effects on this SOL were then measured at the function of the drug dose, generating one dose-response curve for all doses of drug. In comparison, the switch-off paradigm in the present study measured the SOL as a function of frequency, generating the frequency-response curve for each doses. Consequently, each dose of drug produces frequency-response curve giving a more accurate evaluation of the SOR. In addition to the methodological difference of the switch-off paradigm, the present result firstly demonstrated that non-BZD drug ABC also attenuated the SOR induced by the PAG stimulation.

With the regard of the possible mechanism of the effect of DZP on the SOR, the relevant studies will be described. In punishment procedures, both BZDs and 5-HT antagonists have been shown to increase low rates of punished operant behavior (Graeff, 1974 and 1976; Graeff and Schoenfeld, 1970; McMillan, 1975). In addition, the facilitatory effect of a BZD, oxazepam, on punished operant responding has been proposed to correlate with oxazepam-induced decrease in 5-HT turn-over in the rat

midbrain (Wise et al., 1972). Wise et al. (1972) suggested that the facilitatory action of the BZDs on punished responding may be mediated by a reduction of 5-HT in a behavioral inhibitory system. However, previous switch-off experiments showed that CDP and 5-HT antagonists, cyproheptadine and methysergide, had opposite effects on escape behavior from EBS (Schenberg and Graeff, 1978): low doses of CDP (3 to 10 mg/kg i.p.) increased escape latencies to terminate EBS of the PAG, whereas cyproheptadine and methysergide tended to facilitate escape latencies. Thus, the inhibitory effects of BZDs on escape from EBS cannot be solely explained by impairment of 5-HT transmission. Instead, it was hypothesized that an inhibitory role of GABA/BZD receptor complex mediates that stimulus. This view is supported by the present results with FLU.

Neither FLU alone (0.1, 1, and 10 mg/kg, Figure 9) nor the combined treatment of FLU (10 mg/kg) with DZP (2.5 mg/kg, Figure 10a) or ABC (0.5 mg/kg, Figure 10b) affected the SOL. At the dose used in the present study, FLU behaved as a pure BZD antagonist. It was inactive by itself, but blocked the effects of DZP and ABC on the SOL. Consistently, the DZP-induced increase in the escape latency was reduced by the pretreatment with $GABA_A$ antagonist bicuculline (3 mg/kg, i.p.) (Bovier et al., 1982). There is a previous switch-off experiment (Lloyd, et al., 1981) which

obtained a similar result to the present result. The increased escape latency by DZP (7.5 mg/kg i.p.) was blocked by the pretreatment with FLU (20 mg/kg i.p.). However, the present study is the first to show that FLU dose-dependently (0.1, 1.0 and 10 mg/kg) blocked the effects of DZP and ABC on the SOR.

These behavioral results of FLU agree with a body of neurochemical evidence. *In vivo* FLU behaves as a competitive BZD receptor blocker, since it inhibits [^{3}H] DZP-specific binding and selectively antagonizes central BZD drug actions (Hunkeler et al., 1981). In addition, the effects of CDP (80, 160 and 320 nmol) as well as midazolam (20, 40 and 80 nmol) on the escape threshold following their microinjection into the PAG were blocked by a dose of FLU (80 nmol) which by itself did not modify the threshold when microinjected into the PAG (Audi and Graeff, 1984). In *in vitro* experiments, the binding of radioactively-labeled FLU is not increased by the presence of GABA, in contrast with BZD agonists (Mohair and Richards, 1981). This lack of cooperation with the GABA receptors is proposed to be due to the absence of intrinsic activity of FLU on the GABA receptors (Ehlert et al., 1984).

The results in the present study showing that DZP increased the SOL, and FLU reversed this effect, strengthen the above hypothesis that the effect of DZP on the SOR is mediated by inhibitory GABAergic modulation

in the negative reinforcing brain stimulation. There are more data further supporting this hypothesis.

1) GABAergic agonists as well as other compounds acting on GABA transmission affect the behavioral responses elicited by EBS. Thus, an i.p. injection of progabide, a GABA agonist, increases the latency to escape from the PAG stimulation (Bovier et al., 1982). Furthermore, microinjections of GABA, CDP or pentobarbital into the PAG were found to raise the escape threshold from the EBS (Brandao et al., 1982). In addition, microinjections of muscimol into the PAG increases SOL in a switch-off paradigm (Di Scala et al., 1984).

2) Microinjection of the GABA antagonist, bicuculline methiodide into the PAG produces a defensive flight behavior (Di Scala et al., 1984), similar to the ones induced by EBS of the PAG. Local pretreatment with THIP, a GABA agonist after microinjection of bicuculline methiodide attenuates this flight behavior (Krogsgaard-Larsen et al., 1977). Similarly, pretreatment with GABA antagonizes the effect of a microinjection of either bicuculline or picrotoxin (Brandao et al., 1982).

Considering the ABC data, the results of the present experiment speak for a greater potency of ABC than DZP on the SOR. ABC increased the SOL at five-fold lower dose than DZP. This is consistent with the previous

studies where ABC showed antipunishment properties in mice at doses 5 to 10 times lower than the equivalent doses of DZP (Stephens et al., 1990). *In vivo* receptor binding studies also showed a higher potency of ABC compared to DZP reflected as a higher affinity of ABC for [^{3}H] BZD labeled receptors (Stephens et al., 1993).

However, ABC is much less potent than DZP in chimney test and muscle relaxation test as measures of motor coordination (Stephens et al., 1990). In that study, mice were administered with either ABC or DZP (dose range for both drugs, 0.1-100 mg/kg) or vehicle i.p. Thirty min later they were introduced singly into one end of a perpex tube. The mice were positioned head down at the bottom of the tube. Control mice easily climbed backward up to a height of 30 cm within 60 sec. The number of mice unable to reach this criterion after drug treatment was noted. The anxiolytic activity of several BZDs correlated with their potency in impairing the ability of mice to escape from a chimney. In contrast to DZP, ABC did not induce an impairment of muscle coordination in this test. In the test of ataxia as a motor coordination test, ABC was ineffective to induce ataxia at doses up to 100 mg/kg (Stephens et al., 1993). The very weak activity in these motor coordination tests indicate a separation of the anxiolytic and the muscle relaxant effects of ABC in contrast to DZP. This was confirmed by behavioral observations in the present experiments. ABC treated rats

did not show any sedation or impairment of muscle coordination even at the high doses. In addition, these results suggest that the switch-off paradigm can detect not only conventional BZD anxiolytics but the partial BZD agonist ABC, which has a less incidence of side effects than BZDs.

Effect of BUS on the SOR

When BUS is given to humans, significant relief of anxiety is obtained only after repeated doses of BUS (Rickels et al., 1982; Jacobson et al., 1985). Thus, the present study examined the chronic and acute effects of BUS on the SOR induced by EBS of the PAG. Acute BUS (1.25, 2.5 and 5 mg/kg) failed to modify the SOL (Figure 12), whereas the highest acute dose (10 mg/kg, Figure 12) or chronic BUS (2.5 mg/kg, twice a day for three days) significantly increased the SOL (Figure 13). Author assumps that the SOR of the highest dose of acute BUS may be an artifact of motoric disturbance and that only a multiple dose regimen of BUS can affect the SOR induced by negative reinforcing stimulation, which is comparable to the clinical observation. This assumption is based on the following logic.

The failure of acute BUS (1.25, 2.5 and 5 mg/kg) to modify the SOR is consistent with the previous data that acute BUS (2.5-20 mg/kg p.o.) did not affect response in the elevated plusmaze test (Wada and Fukuda,

1991). In contrast to BUS, DZP in the same test, increased the percentage of time spent on the open arms and of open-arm entries which is an indice of anxiolytic effect.

Since acutely administered high dose of BUS (10 mg/kg, Figure 12) also increased the SOL, the effects of both regimens of BUS [high dose acute BUS (10 mg/kg) and chronic BUS (2.5 mg/kg)] on the SOL were assessed at a high frequency of 100 Hz. This test was done to examine whether the increased SOL induced by this drug was due to an impaired motoric capacity to perform the SOR. Had any motoric disturbances occurred, the rat would not be able to perform SOR as fast as he should at high frequency, resulting in longer SOL than that of control condition. As a result, the increased SOL followed by acute high dose (10 mg/kg) of BUS remained significantly higher (at 100 Hz) than that of control condition (at 70 Hz) (Figure 14b). For chronic BUS, however, the increased SOL could be reversed to the baseline level-SOL when the frequency was increased to 100 Hz (Figure 14a). This result suggests that the increased SOL by an acute high dose BUS (10 mg/kg) is unlikely to be due to attenuation of NRS, but perhaps due to nonspecific motoric inhibition. To interpret the present results of BUS, the behavioral effects of BUS in the punishment paradigm and feasible mechanisms of BUS needs to be considered.

There are three major 5-HT projections to the forebrain (Iversen, 1984). A dorsal pathway arising from the medial/rostral nuclei raphe dorsalis and innervating the caudate/putamen; a medial pathway from the nuclei raphe dorsalis to substantia nigra and a ventral pathway arising from the mesencephalic raphe nuclei to innervate the interpeduncular nucleus, ventral tegmentum, thalamus, hypothalamus, amygdala, cortex and hippocampus. Substantial experimental literature exists implicating that the 5-HT pathway from the raphe to the nigrostriatal regions inhibits general motor behavior (Carter and Pycock, 1979; Thiebot et al., 1984). Selective lesions were made by a neurotoxin of 5-HT transmitter, 5,7-DHT in the substantia nigra to destroy its innovation. Such a lesion resulted in a release of foot-shock punished responding in the punishment task (Carter and Pycock, 1979), and in the conditioned emotional procedure (CER) (Thiebot et al., 1984). In the CER, a rat was trained to press a lever for food and then periodically a sound occurred, followed, after a brief delay, by shock. After experiencing repeated pairing of the sound and the shock, rats came to inhibit responding at the onset of the sound and only began responding for food again when the danger of the shock had passed. Sepinewall and Cook (1978) also showed that 5-HT antagonists cinanserin, cyproheptadine, methysergide and lysergic acid diethylamide released response suppression in the rat in the CER. Depletion of 5-HT in

brain with ρ-chloroamphetamine also produces large increases in punished responding (Prarr et al., 1979).

Thiebot et al. (1984) implied that this response release is part of a general motoric disinhibition and suggested that the 5-HT projection from the dorsal raphe to the substantia nigra may play a role in the response suppression induced by punishment. This view is supported by the following results (Iversen, 1984): 1) 5-Hydroxy-tryptophan causes a general suppression of behavior in the pigeon. 2) 5-HT when infused into the ventricles, suppresses self-stimulation in the rat.

Other studies showed the contradictory role of 5-HT in the behavioral suppression (Graeff, 1974 ; Graeff and Rawlins, 1980). Rats were trained to press a lever, and then punished by foot-shock or by dorsal PAG stimulation (Graeff and Rawlins, 1980). Control rats elicited decreased lever-pressing. Destroying 5-HT neurons by septal lesions disinhibited lever-pressing behavior punished by foot-shock, but did not increase comparable response rates punished by dorsal PAG brain stimulation. Previously, Graeff (1974) has obtained the same results with 5-HT antagonists: methysergide and cyproheptadine released foot-shock punished responding whereas failed to release the punished responding by dorsal PAG stimulation. Thus, 5-HT mechanisms do not seem to play a

major role in the suppression of responding produced by dorsal PAG stimulation.

Comparably, metergoline, a 5-HT receptor blocker, apparently enhanced subjective anxiety in healthy volunteers, in spite of causing major increases in punished responding in pigeons (Schenberg and Graeff, 1978). In a agreement, parachlorphenylalanine (serotonin-depleting drug) has disinhibiting effect (Graeff, 1974) in conflict procedures, although this drug lacks an anxiolytic effect in man (Weissman, 1973). Thus, it is unlikely that the ascending 5-HT systems from the raphe region to forebrain are major neural substrates accounting for the NRS of the EBS. It is proposed that, in punishment tests, both the behavioral inhibition system and the negative reinforcing system act together to produce response suppression. In addition, septal brain lesion and 5-HT antagonists would preferentially affect the behavioral inhibition system, while BZD drugs would preferentially impair the negative reinforcing system. Blanchard et al. (1970) have similarly suggested that the fear response has both an avoidance and an immobility component, only the latter being impaired by septo-hippocampal lesions.

The behavioral effects of BUS have been variable and smaller than those observed with BZDs (Cook and Davidson, 1973) in the punishment procedures. BUS produced dose-related increases in punished behavior

of pigeons (Witkin et al., 1987). Conversely, in rats and squirrel monkeys, the characteristic increases in punished responding obtained with BZDs are not consistently reported for BUS (Weissman et al., 1984).

BUS is known to have affinity for 5-HT_{1A} receptors, and there is evidence that increases in punished responding produced by this compound in pigeons are due to activity at these receptors (Witkin et al., 1987). In that experiment, responses were maintained under fixed-ratio schedules of food presentation; every 30th response produced a brief electric shock and suppressed responding. BUS produced dose-related increases in punished responding. A 5-HT agonist, MK-212, antagonized whereas the 5-HT antagonist, cyproheptadine, potentiated the effects of BUS. In addition, BUS displaced [^{3}H]-8-OH-DPAT (a selective 5-HT_{1A} agonist) from both pigeon cerebrum and rat hippocampal membranes. If we assume that the relevant 5-HT_{1A} receptors are located presynaptically (Goodwin et al., 1985), then an agonist action of acute BUS would reduce 5-HT transmission. Thus, BUS disinhibits response, consistent with previous reports in the punishment procedure. However, the failure of BUS and 8-OH-DPAT to increase the punished response in rats remains to be explained. Interestingly, the number of binding sites for [^{3}H]-8-OH-DPAT in the pigeon cerebrum was 25 to 50% lower than those found in the rat brain.

These findings may imply that the different effects of BUS in rats and pigeons could result from the differences in the species.

With the above reports in mind, the suppressive effects of acutely administered high dose BUS on the switch-off response can be interpreted as follows: high dose of acute BUS nonspecifically binds to 5-HT receptors located presynaptically and postsynaptically. It consequently results in global motoric inhibition. Interestingly, pretreatment with acute BUS (5 mg/kg) tended to decrease the SOL, even though it was not statistically significant. This could imply that occurs presynaptic binding of acutely treated BUS (5 mg/kg) at the 5-HT_{1A} receptors, which antagonizes the 5-HT function, thus resulting in motoric disinhibition. There are data consistent with these results: 1) The turnover of 5-HT in the central nervous system has been reduced by acute BUS (Mennini et al., 1986). 2) 5-HT_{1A} receptors, which exist presynaptically (Verge et al., 1985), and non-5-HT_{1A} receptors, which exist postsynaptically, may play opposite roles in mediation of their action perhaps due to different locations in the CNS (Jenck et al., 1989).

Conversely, the effect of chronic BUS on the SOR did not appear to be a motoric consequence of the drug since the increased latency induced by chronic BUS (2.5 mg/kg twice a day for three days) was decreased to the base-line level by increasing the frequency. From a neurochemical point of

view, this may be due to receptor changes induced by chronic treatment of BUS to exhibit its action different than 5-HT induced behavior-inhibitory system.

In this context, one of the most plausible explanations comes from the previous data (Gobbi et al., 1991) which demonstrated the involvement of BZD/GABA system following chronic regimen of BUS in its anxiolytic action. Indeed, given the antianxiety effect of BUS, its effect on brain BZD receptors was recently researched. Using receptor autoradiography, the affinity of BUS and 1-PP, a main metabolite of BUS, for 5-HT_1 and BZD receptors was evaluated (Gobbi et al., 1991). In the substantia nigra, BUS indirectly affected [^{3}H] BZD binding; a decrease was observed after acute treatment, whereas chronic treatment resulted in increased binding.

In another experiment, BUS (10^{-5} M) *in vitro* had no effect on the binding of [^{3}H] DZP or [^{3}H] flunitrazepam for the BZD receptors However, when given *in vivo*, BUS at an oral dose of 10 mg/kg increased the binding of [^{3}H] DZP in the cerebellum by 40 % (Garattini et al., 1982). Likewise, some BZDs, such as estazolam and tofisopam (Mennini and Garattini, 1983), increased [^{3}H] DZP binding *in vivo* in the same brain area.

Because of the close relationship between BZD receptors and GABA receptors, the activity of BUS on the latter was assessed. BUS did not

displace GABA binding (Garattini et al., 1983). However, on washed cerebellar membranes in rats, BUS (10^{-7}M), increased GABA binding (Garattini et al., 1983).

Recalling the present data, it was shown that BZD agonist DZP and chronic BUS resulted in the same effects on the SOR. In addition, it appeared that the blockade of the SOR induced by chronic BUS is unlikely to be due to 5-HT modulation of behavioral inhibition. Thus, these results and the previous review give rise to the hypothesis that there may be a common mechanism between the action of BUS and GABA/BZD agonists in modulation of NRS. The mechanisms by which chronic BUS leads to such modifications of $GABA_A$/BZD receptors and 5-HT receptor remain to be further determined. Nevertheless, the results with BUS in the present study may offer a step to identify a possible common pathway in the mechanism of action of BZDs and non-BZD anxiolytic drugs.

Effects of PTZ on the SOR

The SOL following pretreatment with PTZ (5, 10 or 20 mg/kg, 10 min, Figure 15) decreased in a dose related manner, suggesting that PTZ potentiated the NRS of the stimulation. This is comparable to the discrimination data, where PTZ potentiated the discriminative cue of the brain stimulation. There is analogous data which show a decreased SOL by drug pretreatment (Leidenheimer and Schechter, 1988). BZD receptor

inverse agonist FG 7142, which induces anxiety in humans (Corda et al., 1983; Scala et al., 1982; Dorow et al., 1983), shortened the SOL in rats stimulated at the MLR in the switch-off paradigm. However, the present study is the first to show that PTZ potentiates the NRS induced by EBS of the PAG. The facts that both drugs, FG 7142 and PTZ induce anxiety in men (Corda et al., 1983; Scala et al., 1982; Dorow et al., 1983), and decrease the SOL lead the following suggestion: The SOR induced by negative reinforcing brain stimulation of the PAG can be useful for screening PTZ-like anxiogenic drugs. In addition, the report that PTZ antagonizes the regulatory sites of GABA/BZD receptor complex (Simmonds, 1983) strengthens the hypothesis: The action of BZDs on the negative reinforcing system is mediated by the GABA/BZD receptors.

In summary, anxiolytics DZP, ABC (putative anxiolytic) and chronic BUS and an anxiogenic, PTZ showed opposite effects on the SOR in the present paradigm. DZP, ABC and chronic BUS attenuated whereas PTZ potentiated the SOR induced by the negatively reinforcing PAG stimulation. These data support the argument that the SOR can be extended to detect non-BZD anxiolytics as well as PTZ-like anxiogenics. One step further, the results with BZDs (DZP and ABC) and 5-HT_{1A} drug (chronic BUS) propose the hypothesis that there is a possible common pathway between GABA/BZD receptors and 5-HT neurotransmission modulating NRS induced by PAG stimulation.

Conclusion

Using electrical brain stimulation of the PAG and the MH, these results demonstrate the following.

1) EBS of the PAG and the MH produce a discriminative stimulus in a two-lever food-reinforced discrimination paradigm. EBS of the PAG also produces a NRS in a switch-off paradigm. These stimulus effects covary with the strength of the EBS.

2) PTZ potentiates both a discriminative stimulus and a NRS effects whereas DZP and ABC attenuate a NRS effects. These anxiolytics-induced effects on the SOR are likely due to the attenuation of the NRS rather than a general motoric inhibition. DZP failed to block the discriminative stimulus, perhaps due to the fact that the low stimulation intensities were not strong enough to serve as a distinct stimulus effects.

3) FLU blocked the effects of DZP and ABC on the SOR, suggesting that GABA/BZD receptors mediate the NRS effect.

4) Acute BUS did not modify whereas chronic BUS attenuated the NRS effect. Chronic BUS may induce neurochemical changes presumably, in a common pathway between GABA/BZD and 5-HT.

5) Thus, the use of switch-off paradigm can be extended to detect novel anxiolytics, ABC (putative anxiolytic) and BUS as well as an anxiogenic, PTZ. In addition, the use of NRS in a switch-off paradigm can more reliably detect both

anxiolytic and anxiogenic drugs than the use of discriminative stimulus in a two-lever food-reinforced discrimination paradigm under the condition of this study.

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