The Role of the Dopaminergic System in the Production of Ultrasonic Calls in Adult Rats

by

Amanda J. Wintink

Submitted in partial fulfilment

of the requirements for the degree of

Master of Arts

Department of Psychology

BROCK UNIVERSITY

St. Catharines, Ontario

July 2000

Dedicated to my Grandma (Jessie) and Gigi (Joseph) Wintink
for their support, encouragement, and love.

26 July 2000

Summary

Ultrasonic vocalizations (USV) are emitted by rats in a number of social situations such as aggressive encounters, during sexual behavior, and during play in young rats, situations which are predominantly associated with strong emotional responses. These USV typically involve two distinct types of calls: 22 kHz calls, which are emitted in aversive situations and 50 kHz calls, which are emitted in non-aversive, appetitive situation. The 50 kHz calls are the focus of the present study and to date both the glutamatergic and the dopaminergic systems have been independently implicated in the production of these 50 kHz calls. The present study was conducted to examine a possible relationship between glutamate (GLU) and dopamine (DA) in mediating 50 kHz calls. It was hypothesized that the dopaminergic system plays a mediating role in 50 kHz calls induced by injections of GLU into the anterior hypothalamic/preoptic area (AHPOA) in adult rats.

A total of 68 adult male rats were used in this study. Rats' USV were recorded and analyzed in five experiments that were designed to test the hypothesis: in experiment 1, rats were treated with systemic amphetamine (AMPH) alone; in experiment 2, intra-AHPOA GLU was pretreated with systemic AMPH; in experiment 3, intra-AHPOA GLU was pretreated with intra-AHPOA AMPH; in experiment 4, rats were treated with high and low doses of intra-AHPOA AMPH only; in experiment 5, rats were treated with systemic haloperidol (HAL) as a pretreatment for intra-AHPOA GLU.

Analysis of the results indicated that AMPH has a facilitatory effect on 50 kHz USV and that a relationship between DA and GLU in inducing 50 kHz calls does exist. The effect, however, was only observed when DA receptors were antagonized with HAL and was not seen with systemic AMPH pretreatments of intra-AHPOA GLU. The DA-GLU relationship at the AHPOA was unclear.

Acknowledgments

I would like to thank my advisor, Dr. Stefan Brudzynski, for his time and energy devoted to this thesis and also for the many insightful ideas he has shared with me.

Thanks also to Drs. Sid Segalowitz & Jane Dywan for their wonderful advice and feedback, and especially to Dr. Segalowitz for feeding my intellectual curiosity.

I would also like to acknowledge my outer-Ontario support system throughout these past two years including my partner Dan McConnell, my colleagues of ΨΣΠ, a.k.a Smarty Pants (Lisa Fiorentino, Marsha Penner, and Sandra Wiebe), and particularly Dr. Katherine Schultz, from the University of Winnipeg, for equipping me with a foundation of skills necessary to succeed at the graduate level. Thanks also to Nancy Sancartier for being a great lab-mate and culturing me with Niagara wine!

* This study was supported by an NSERC operating grant to Dr. Stefan M. Brudzynski.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AP5:	Antagonist of the glutamate receptor sub-type NMDA
AMPA:	Glutamate receptor sub-type named after its primary agonist. Same receptor site as QA.
AMPH:	amphetamine
AHPOA:	anterior hypothalamic/preoptic area
Ctrl/Glu:	control i.p, injection pretreatment with i.c. glutamate
DA:	dopamine
GDEE:	Antagonist of the glutamate receptor sub-type AMPA
GLU:	glutamate
KA:	kainate acid, an agonist of the glutamate sub-receptor kainate.
LoAmph/Sal:	1.5 mg/kg of i.p. amphetamine pretreatment with i.c. saline treatment
LoAmph/Glu:	1.5 mg/kg of i.p. amphetamine pretreatment with i.c. glutamate treatment
LDT:	laterodorsal tegmentum
HAL	Haloperidol
Hal/Glu:	2.0 mg/kg of i.p. haloperidol pretreatment with i.c. glutamate treatment
HiAmph/Sal:	2.5 mg/kg of i.p. amphetamine pretreatment with i.c. saline treatment
HiAmph/Glu:	2.5 mg/kg of i.p. amphetamine pretreatment with i.c. glutamate treatment
HPOA:	hypothalamic preoptic area
i.c.	Intracerebral
i.p.	Intraperitoneal
MdAmph/Glu:	2.0 mg/kg of i.p. amphetamine pretreatment with i.c. glutamate treatment
MdAmph/Sal	2.0 mg/kg of i.p. amphetamine pretreatment with i.c. saline treatment
MFB:	Medial Forebrain Bundle
MK-801:	NMDA receptor sub-type antagonist
N.Acc.:	Nucleus accumbens
NMDA:	N-methyl-D-aspartate. A glutamate receptor sub-type named after its primary agonist.
QA:	Quisqualate. A glutamate receptor sub-type named after its primary agonist, quisqualate. Same receptor sites as AMPA.
Sal:	saline
Sal/Glu:	i.p. saline pretreatment with i.c. glutamate treatment
Sal/Sal	i.p. saline pretreatment with i.c. saline treatment
USV:	ultrasonic vocalizations
VTA:	ventral tegmental area

Introduction

Ultrasonic Vocalizations and Behavioral Context

Ultrasonic vocalizations (USV) are emitted by rats in a number of social situations including fighting behavior (Sales & Pye, 1974), defensive behavior (Sales & Pye, 1974), during and in anticipation of play (Knutson, Burgdorf, Panksepp, 1998), during various sexual behavior stages (McIntosh & Barfield, 1980), and as predatory alarm calls in rat colonies (Blanchard, Blanchard, Agullana, & Weiss, 1991). These are situations which are biologically important and are predominantly associated with strong emotional responses. These USV typically involve two distinct call patterns in adult animals: calls of an approximate sound frequency of 18 - 32 kHz, known as '22 kHz calls', which are emitted in aversive situations and calls of an approximate sound frequency of 35 - 70 kHz, known as '50 kHz calls', which are emitted in non-aversive, appetitive, social situations. Because there is strong consensus that the 22 kHz calls reflect responses in aversive situations, 22 kHz can be used as a measure of anxiolytic effectiveness. Thus, a future aim in researching the nature of the 50 kHz calls is to also provide information that could potentially shed light on animal emotionality and hence provide a model on which mood drugs could be tested.

The 22kHz calls have been reported to occur when the rat is confronted by a predator (Blanchard, Blanchard, Agullana, & Weiss, 1991), in response to handling and touch of naive rats (Brudzynski & Ociepa, 1992), when vigorously shaken (Thomas & Barfield, 1985), and in the presence of an aggressor (Corrigan & Flannelly, 1979). These are situations which are dangerous and predominantly stress-invoking for the rat. Calls of the 50 kHz type are emitted during different situations, for example, when the rat is in the presence of another anaesthetized rat (Blanchard, Yudko, Blanchard, & Taukulis, 1993), in environments which have previously been associated with reward (Knutson, Burgdorf, & Panksepp, 1999), when reunited with a littermate (Blanchard, in prep), and in

anticipation of and during play in young rats (Knutson, Burgdorf, & Panksepp, 1998). It seems, therefore, that 22 kHz and 50 kHz calls represent two different behavioral states; aversive and appetitive/non-aversive. While 22 kHz calls have been studied in a number of aversive situations they have not been reported in non-aversive situations. The 50 kHz calls have only been reported in a few situations which have been primarily non-aversive, appetitive, social interactions.

Twenty-two kHz calls have been thoroughly examined in rats. Nonetheless, debate over the nature of the 22 kHz calls still exists with some researchers arguing that the calls represent a communicatory signal (Blanchard et al. 1991; Blanchard et al., 1993; Brudzynski, 1994; Brudzynski, Bihari, Ociepa, & Fu, 1993; Brudzynski & Chiu, 1995) and others arguing that the calls result as an acoustic by-product of a respiratory mechanism to increase gas exchange in the lungs (Blumberg & Alberts, 1991; Blumberg, Sokoloff, Kirby, & Kent, 2000). Fifty kHz calls have been less well studied and the debate is based on different issues. Initially, Blumberg (Blumberg & Alberts, 1991) stated that the 50 kHz calls have a different production mechanism from the 22 kHz calls but later argued that the 50 kHz calls are a result of thoracic compression precipitated by heightened locomotor activity (Blumberg, 1992). Previously, Bell (1974) proposed that 50 kHz calls, and 22 kHz calls, are produced by arousal and are arousal-producing for nearby conspecifics, and that changes in acoustic parameters reflect differences in the energy used to emit the signals. Others have provided evidence against a general arousal theory by dissociating locomotion and activity from a rewarding situation, and concluded that 50 kHz calls reflect appetitive-motivational behavior rather than general arousal (Burgdorf et al., 2000; Knutson et al. 1998).

Thus, the literature strongly supports a contextual distinction between 50 kHz and 22 kHz calls reflecting differences in behavioral significance. The 22 kHz calls are present in stressful, aversive situations whereas the 50 kHz calls are present in positive,

social situations. Also, the 22 and 50 kHz calls differ in acoustic parameters other than sound frequency. For example 22 kHz calls have a narrower bandwidth while 50 kHz calls have larger variations in bandwidth, and the single call duration is much longer for 22 kHz calls compared to 50 kHz calls (see Table 1). Because it is clear that the calls are different acoustically and behaviorally, it is highly likely that there are different neural mechanisms underlying the production of these calls. Some neurochemical and neuroanatomical differences have already been examined.

Neurochemical Correlates of USV

The distinction between the two types of calls is also evident from neurochemical studies in which the two types of calls have been elicited using different pharmacological agents. The 22 kHz calls can be induced with carbachol, a predominantly muscarinic agonist of the cholinergic system (Brudzynski, 1994), by inhibiting the neurons in the mediobasal hypothalamic-preoptic area (HPOA) (Brudzynski, Kadishevitz, & Fu, 1998; Brudzynski, McLachlan, & Girvin, 1991). The 50 kHz calls, however, are triggered by stimulating the same area with glutamate (GLU) (Fu & Brudzynski, 1994) which has excitatory effects on the same neurons (Brudzynski et al., 1998). Both types of calls are prevented by treatment with the respective antagonists; for example MK-801 antagonized the effects of GLU (Fu & Brudzynski, 1994) whereas scopolamine (Brudzynski & Barnabi, 1996) and atropine (Brudzynski, 1994) antagonized the effects of carbachol. It was also shown that GLU does not elicit 50 kHz calls when injected into an area outside the mediobasal HPOA, such as the laterodorsal tegmental nucleus (LDT) or the parabracial nucleus (PBN), although GLU, used as a neuronal stimulant, did elicit 22 kHz calls when injected into the LDT but not the PBN (Brudzynski & Barnabi, 1996).

Triggering and inhibiting calls with agonists and antagonists has shown that the 22

kHz calls are largely mediated via the cholinergic system, and are acoustically indistinguishable from naturally occurring calls (Brudzynski, Ociepa, & Bihari, 1991) whereas the 50 kHz calls are mediated via the glutamatergic system; the mediobasal HPOA is involved in both systems. Also, with GLU injections into the anterior HPOA – a region of the mediobasal HPOA – sound frequency increased with increasing doses of GLU (Fu & Brudzynski, 1994). Taken together, it is obvious that 22 and 50 kHz calls are unequivocally distinct from each other differing acoustically, neurochemically, and in the behavioral contexts under which they are emitted. The 22 kHz calls are emitted during periods of stress, likely as a warning signal, are acoustically comparable between naturally occurring and pharmacologically induced calls with a narrow range of single call sound frequency, and are mediated via the cholinergic neurochemical system. The 50 kHz calls, in comparison, are emitted during periods of social, appetitive, motivational states, have a greater range of single call sound frequency, and are mediated via the glutamatergic system. The distinction between the two types of calls is important for the present study which is designed as an attempt to further understand the neurochemical mediation responsible for production of 50 kHz calls.

50 kHz Calls and Appetitive, Rewarding Situations

In addition to the known mediation of GLU in the production of 50 kHz calls, the dopaminergic system has also been implicated in this type of call because 50 kHz calls have been emitted during appetitive, rewarding situations (for example, Knutson, Borgdorf, & Panksepp, 1999) and because dopamine (DA) is well known for its role in reward (see Engel, Oreland, Ingvar, Pernow, Rossner, Pellborn, 1987). However, little direct evidence exist for this connection. It was only recently that a relationship between DA and 50 kHz calls was reported. The link between DA and USV was observed earlier in the production of 22 kHz calls when administration of haloperidol (a DA antagonist)

was shown to reduce the number of distress calls in young rat pups removed from the warmth of their nest and mother (Cagiano, Sales, Renna, Racagni, & Cuomo, 1986). However, these early results are difficult to interpret because the rat pups were administered prolonged treatments of haloperidol and the authors suggest that the results may be due to an impaired functional maturation of the catecholaminergic system. They are also in contrast to later results where in adult rats where it was found that DA D2-like receptor sub-type agonists (for example, apomorphine and quinpirole), and not D1-like agonists, dose-dependently reduced 22 kHz calls, which suggests an anxiolytic effect of DA (Bartoszyk, 1998). Also, haloperidol and other DA antagonists, in contrary, did not have an inhibitory effect on the calls in this study, hence, supporting the action of DA on 22 kHz calls.

More indirect evidence emanates from the reward conditioning literature. Reward is normally measured in terms of place conditioning (i.e. conditioning to the environment associated with a previously received reward) and intracranial self-stimulation (i.e. bar-pressing behavior which initiates the delivery of a reward, such as DA agonists or electrical stimulation, directly to an area of the brain, for example, the nucleus accumbens (N.Acc) (for a review see McBride, Murphy, & Ikemoto, 1999). The association between DA and 50 kHz calls was partially shown by Knutson et al. (1999) who showed that rats vocalized more in an experimental chamber which was associated with the rewarding effects of previously administered amphetamine (a dopaminergic agonist which increases the release and effects of DA). However, the researchers did not measure the number of calls emitted after the injections of amphetamine (AMPH) itself, so although it was assumed that the calls were emitted due to the conditioning of a previous reward, it could not be determined whether calls would be emitted under direct influence of AMPH itself. The authors did previously report that AMPH increased the number of 50 kHz calls when administered systemically (Knutson, Burgdorf, & Panksepp, 1997) and when injected

directly into the N.Acc. (Burgdorf, & Panksepp, 1999); however, both sets of results have not yet been published. The distinction between calls emitted during a conditioned response and those emitted under the influence of a psychostimulant is important in understanding the role of DA in eliciting 50 kHz calls. At present, it is assumed that 50 kHz calls are emitted by rats in response to a rewarding situation but it may be that DA itself acts directly to elicit 50 kHz calls such that 50 kHz calls and rewarding experiences may occur by parallel processes rather than serial processes.

Knutson, Burgdorf, and Panksepp (1998) also conducted a series of experiments examining the 50 kHz calls emitted by juvenile rats during the social interactions of play and the anticipation of play. In the first experiment they demonstrated that rats emit 50 kHz calls when playing with another rat compared to when rats were placed in a similar cage without a playmate. In the following experiment rats were allowed to play or were screened off from the playmate for two days in a counterbalanced order. The rats which played on Day-1 and then were screened off from the playmate on Day-2 called more during the screened-off session compared to the play session. These rats also called more than the rats which were screened off first and then allowed to play and more than the rats allowed to play on both days or were separated on both days. Thus, the session in which the most calls were emitted was when the rats which had previously played were subsequently screened off. It was also only in the conditions in which play either preceded or followed a separation that the number of calls increased from Day-1 to Day-2. The authors also demonstrated that rats called more when placed in a play chamber without the presence of the playmate after 3 days of prior play sessions than when placed in a chamber not associated with play. Finally, rats which had been housed in single cages called more during play than rats which had been housed in pairs which the authors attributed to more play motivation of the isolated rats. The results were interpreted as 50 kHz calls signifying appetitive behavior exhibited in either anticipation of play or play motivation.

In another study, it was also reported that anticipation of rewarding brain stimulation to the ventral tegmental area (VTA), the originating cell group of the DA reward system (see below), induced 50 kHz calls in adult rats (Burgdorf, Knutson, & Panksepp, 2000). Furthermore, this effect was also seen with stimulation to the lateral hypothalamus and was not a function of locomotor behavior. Also, more 50 kHz calls, and no 22 kHz calls, were emitted during cue exposure without the rats being allowed to self-stimulate. The authors noted that cue exposure was different from 'frustrative non-reward'. Frustrative non-reward, by definition, would occur after rats had learned to self-stimulate and then during a subsequent attempt to self-stimulate no rewarding stimulation would occur. In the study by Burgdorf et al. 22 kHz calls were emitted during frustrative non-reward whereas no 22 kHz calls were emitted during cue exposure nor were there any emitted during the actual brain stimulation periods. Although 50 kHz calls were present during the frustrative non-reward period, the call number was significantly reduced. The authors also reported that the 50 kHz calls are not only induced by electrical brain stimulation but also by natural rewards such as food anticipation, which they demonstrated by conditioning the animals to the time of food arrival and measuring the number of 50 kHz calls. Thus, they concluded that their results support the notion that 50 kHz calls reflect appetitive behaviors such as reward self-stimulation and reward anticipation and are non-aversive.

Neuroanatomy of DA and GLU Pathways, and Hypothalamic-Preoptic Areas

Mesolimbic System (see Figure 1)

The mesolimbic system contains a major dopaminergic cell group and its projections and is associated with the DA reward hypothesis (Fibiger & Phillips, 1987). The mesolimbic (a.k.a. mesocorticolimbic, for example in Leshner & Koob, 1998 when cortical areas are considered) brain system contains a high concentration of dopaminergic

pathways (see Feldman, Meyer, & Quenzer,1997). The dopaminergic pathways originate in the VTA with smaller contributions from the substantia nigra (SN). VTA and the other contributions pass through the medial forebrain bundle (MFB) to innervate the septum, amygdala, hippocampus, nucleus of the diagonal band, anterior olfactory nucleus, and limbic cortical areas (mesocortical components) and ascend to the N.Acc.[1] and the olfactory tubercle (see Figure 1a). Neurons within the MFB are thought to transynaptically activate the ascending DA system (Bozarth, 1987) and project to the lateral hypothalamus and the preoptic nucleus (Kandel, Swartz, Jessell, 2000).

Glutamate

The GLU pathways and receptor densities are described in Cotman and Monaghan (1987). Pathways using GLU include projections from the cortex to the striatum and the hippocampus, the intrahippocampal mossy fiber system and Schaffer collaterals, the dorsal root evoked-monosynaptic response, the lateral olfactory tract, and the primary afferents to the trigeminal nucleus. GLU has three major receptor sub-types which bear the name of their respective major agonists[2]; NMDA, AMPA or quisqualate (QA), kainate acid (KA) and metabotropic. The elicitation of 50 kHz calls in rats was observed using NMDA agonists and subsequently inhibited using NMDA antagonists. There are high densities of NMDA receptors in the N.Acc and slightly lower concentrations in the striatum. There are high levels of binding throughout the olfactory regions and the septum, with lower levels in the bed nucleus of the stria terminalis which are higher than in the regions of the preoptic area. Although it is important to note where the receptors are abundant, the differences in local densities of receptors do not necessarily inform us about their functional importance.

Hypothalamic/Preoptic Area (HPOA)

The mesolimbic system and the HPOA may be functionally linked in the initiation of 50 kHz calls. An anatomical relationship does exist between the HPOA and the

mesolimbic system. For example, lesions to the medial-hypothalamic region of the MFB caused an extensive degeneration of the DA nerve terminals in the N.Acc and the olfactory tubercle (i.e. the termination sites of the mesolimbic system) (Ungerstedt, 1971). The consequences of these hypothalamic lesions implies that a functional relationship exists between medial hypothalamic areas and the dopaminergic reward system.

Calls of 50 kHz have been induced with GLU stimulation to the HPOA (Fu & Brudzynski, 1994) and with AMPH into the N.Acc. (Burgdorf & Panksepp, 1999). Because GLU also acts at the HPOA through excitation (Fu & Brudzynski 1994) to induce vocalizations, and because the calls have already been shown to reflect positive appetitive behavior (Burgdorf et al., 2000; Knutson et al., 1999), one could speculate that projections from the VTA may innervate regions of the HPOA as well as terminating in the N.Acc. However, in order to begin to understand the system mediating 50 kHz calls, the nature of the DA-GLU relationship must first be elucidated. The present study was designed, therefore, to first determine whether DA acts to increase the number of GLU-induced calls emitted and whether the relationship is mediated through the HPOA.

DA-Dependent Behaviors and the DA-Glu Relationship

Locomotor activity, behavioral sensitization, stereotypy, and conditioning are behavioral phenomena which are dependent upon DA levels. Dopaminergic projections activate the caudate-putamen (CP)[1] resulting in stereotypy (see Ellinwood & Balster, 1974; Schiorring, 1979) characterized by excessive rearing, head movements side-to-side, and repetitive/perseverative behaviors, whereas dopaminergic stimulation of the N.Acc. results in locomotor behavior (Brudzynski & Mogenson, 1985; Pijenburg, Honig, Van Der Heyden, & Van Rossum, 1976) as well as conditioned reward. Pharmacological and behavioral sensitization result with repeated administration of, for example, AMPH or other dopaminergic agonists/psychostimulants.

Locomotor Activity

Decreases in locomotor activity, such as freezing responses, can be used as a measure of aversive experience in animals (Brudzynski & Eckersdorf, 1984). Cholinergic agonists injected into the HPOA can decrease locomotor activity (Brudzynski, McLachlan, & Girvin, 1989); the same agonist, as mentioned above, which increased 22 kHz calls emitted by rats when administered into the HPOA. Thus, both locomotor activity and 22 kHz calls are reliant upon similar neurochemical systems and structures. The effects of DA level alterations on locomotor activity are well exemplified by such diseases as Parkinson's Disease which is characterized by severe motor dysfunction as a result of DA depletion in the basal ganglia[1] (Pinel, 1997). Also, haloperidol (a dopaminergic antagonist) decreases voluntary movement in rats (Drinkenburg, Keith, Sahgal, & Andrews, 1999) and increases muscle tone which serves as a model for Parkinson's disease (for example Lorenc-Koci, E., Wolfarth, S., & Ossowka, K., 1996). Increases in DA levels, using stimulants such AMPH, are also known to increase locomotor behavior (Feldman, Meyer, & Quenzer., 1997, Ch. 13).

The co-mediating role of GLU and DA in locomotion has been established in a number of studies. For example, GLU NMDA receptor antagonists block the locomotor stimulating effects induced by cocaine in both rats and mice (Karler & Calder, 1992). Others have argued that the glutamatergic effects on locomotor activity stimulated by DA agonists, such as AMPH, are only mediated through specific GLU receptors such as the AMPA receptor sub-types and not those specific to NMDA receptor sub-types (Burns, Everitt, Kelley, & Robbins, 1994; Freed & Cannon-Spoor, 1990). This was concluded based on a series of experiments which showed that only agonists of the AMPA receptor sub-types (AMPA and QA) potentiated AMPH-induced locomotor activity, while AMPA and NMDA receptor antagonists (CNQX and AP5) both blocked the effects (Burns et al., 1994). Similarly, GDEE, another AMPA antagonist, also blocked AMPH-induced

locomotor activity while NMDA receptor antagonist, MK-801 did not (Freed & Cannon-Spoor, 1990). Hoffman (1994) also found no blocking effect of MK-801.

GLU antagonists alone have also been reported to increase locomotor activity, for example AP5 (Kelley & Throne, 1992) and MK-801 (Hoffman, 1994), as do some agonists, for example, NMDA (Burns et al., 1994). Also, the long-term blocking effects of MK-801 continue to have a reducing effect on rats' activity compared to controls after 10 days from the last exposure (Stewart and Druhan, 1993).

Wolf and Khansa (1991) report that on the first day of treatment there was no additive effect of MK-801 and AMPH on locomotor activity over and above the effects seen after each drug alone, which they argue could reflect a common locomotor stimulation mechanism between GLU and DA. Although discrepancies exist in this literature, the point remains that both DA and GLU have mediating roles in locomotor activity and that they could be part of a common mechanism. Discrepancies in the literature could be explained, for example, by differences in the behavioral state of the animal of the time of testing. For example, the DA agonist quinpirole, injected into the N.Acc. increased activity when the initial activity level was low and decreased activity when the initial activity level was high (Wu, Brudzynski, & Mogenson, 1993). However, regardless of the issues involved in the neurochemical mediation of locomotor activity, the important point remains that a DA-GLU relationship does exist for mediating locomotor activity.

Behavioral Sensitization

The effect of MK-801 in blocking AMPH sensitization was determined through a series of experiments examining both sensitization and conditioning (Stewart & Druhan, 1993). In the first experiment, MK-801 successfully blocked sensitization – measured by administering a lower dose of AMPH than previously received and recording activity. In a following experiment, MK-801 continued to block sensitization irrespective of any

conditioning effects associated with pre-exposures to AMPH. Wolf and Khansa (1991) demonstrated that daily treatments of AMPH paired with MK-801 blocked the development of sensitization-induced locomotor behavior resulting from repeated AMPH administrations. The authors also reported that locomotor activity was not reduced prior to sensitization with MK-801.

Stereotypy

Dopaminergic projections activate the caudate-putamen resulting in stereotypy (see Ellinwood & Balster, 1974; Schiorring, 1979); characterized by excessive rearing, head movements side-to-side, and repetitive/perseverative behaviors (Antoniou, Kafetzopoulos, Papadopoulou-Daifoti, Hyphantis, & Marselos, 1998). There have been a few accounts of stereotypy being blocked by GLU manipulations. For example, stereotypy resulting from cocaine (a psychostimulant similar in effects to AMPH acting on the dopaminergic system) was blocked by three different NMDA antagonists (Karler & Calder, 1992). Cocaine-induced stereotypy was significantly reduced 13-20% with NMDA antagonists (MK-801, CPP, and dextromethorphan) compared to an 80% reduction with saline and a 0% reduction with the DA antagonist haloperidol. Similar results were later obtained in a study with AMPH and cocaine, again using mice (Karler, Calder, Thain, & Bedingfield, 1995). AMPH- and cocaine-induced stereotypy was blocked by antagonists of DA and of NMDA when administered systemically and directly into the striatum. Also, NMDA agonists alone caused stereotypy which was blocked by a NMDA antagonist, however, was not blocked by a dopaminergic antagonist.

Conditioning

Place-conditioning is said to occur when an animal becomes conditioned to perform reward-type behavior in the absence of the reward in the environment in which the initial reward was administered. AMPH can induce a fourfold increase in conditioned responding (Kelly & Throne, 1992) because of its action on the dopaminergic reward

system. Although there have been reports that the NMDA antagonist MK-801 does not block AMPH-induced place conditioning (Hoffman, 1994), others have shown that a blocking effect does occur with both MK-801 (Stewart & Druhan, 1993) and another NMDA antagonist, AP5 (Burns et al., 1994). AMPA antagonists also block conditioned responding from AMPH as did GLU agonists, such as NMDA, QA, and AMPA (Burns et al., 1994).

Vocalization

Although the exact nature of the DA-GLU relationship in locomotion, behavioral sensitization, stereotypy, and conditioning is ill-defined, a GLU role in DA-dependent behaviors is well-founded. This suggests that the two systems function together to bring about these behaviors. This relationship, coupled with the knowledge that 50 kHz calls are emitted by rats under circumstances which are induced by DA, provides a logical premise to warrant the investigation of the DA involvement in GLU-induced 50 kHz calls. Moreover, as mentioned above (Cotman & Monaghan, 1987), glutamatergic pathways project from the cortex to many subcortical structures, for example, the striatum and to the N.Acc. Thus, because the glutamatergic and dopaminergic systems are functionally related in many behaviors they may also be functionally linked in the regulation of 50 kHz calls. This possible GLU-DA relationship in ultrasonic vocalizations has not yet been investigated. If DA elicits 50 kHz calls similar to those observed after GLU injection into the HPOA, then stimulating the HPOA with DA might also elicit the same calls. If DA does not stimulate calling at the HPOA, but does so when administered systemically, then it might be concluded that DA projections do not directly synapse on cells of the HPOA but rather indirectly act on another cell group which in turn acts on the HPOA. The present study will help elucidate these issues and may establish whether DA is indeed involved in the production of 50 kHz calls, whether a DA-GLU relationship exists, and whether or not these effects are mediated at the HPOA.

Goals of the Study

The present study was an attempt to provide documentation of the relationship between GLU and DA in producing 50 kHz calls by examining the influence of brain dopaminergic manipulations on the number and characteristics of GLU-induced calls. It was hypothesized that AMPH administration prior to GLU administration would augment the number of 50 kHz calls produced by rats in a dose-dependent manner. In order to investigate the dopaminergic involvement, experimental manipulation involved recording changes in the number of 50 kHz calls induced by intracerebral injections of GLU into the anterior HPOA (AHPOA) before and after pre-treatment with systemic or intra-AHPOA AMPH. GLU has been previously shown to elicit 50 kHz calls when injected directly into the AHPOA (Fu & Brudzynski, 1994) in a dose-dependent fashion using doses of 16.9, 33.8, and 67.6 μg in 0.2 μl. Pre-treatment with AMPH will increase dopaminergic transmission and this increase is anticipated to increase GLU-induced calls. To further examine the dopaminergic role in the initiation of 50 kHz calls the effect of a systemic DA antagonist (haloperidol) on GLU-induced calls was examined. By both agonizing and antagonizing DA a broader understanding of the DA-GLU relationship is possible.

The acoustic parameters of the calls were also taken into account to determine whether pharmacological manipulations would not change their acoustic characteristics. A difference in some parameters and not others could suggest that the parameters may reflect different communicatory signals.

Hypotheses

Generally, it was hypothesized that the dopaminergic system plays a mediating role in GLU-induced 50 kHz calls in adult rats. The experiments of this study were designed to test each of the following five hypotheses about the relationship between the glutamatergic and dopaminergic systems in producing short, high-frequency ultrasonic vocalizations:

1. Augmenting dopaminergic transmission with systemic administration of the dopaminergic agonist AMPH would induce 50 kHz calls emitted by adult rats.
2. Augmenting dopaminergic transmission with systemic AMPH prior to a GLU injection into the AHPOA would increase the number of 50 kHz calls induced by GLU alone.
3. Augmenting dopaminergic transmission directly in the AHPOA by intracerebral injection of AMPH prior to a GLU injection into the same site in the AHPOA would increase the number of 50 kHz calls induced by GLU alone.
4. Antagonizing DA receptors through systemic administration of the dopaminergic antagonist haloperidol would suppress the rate of 50 kHz calls emitted by rats.
5. For acoustic parameters, single call duration and bandwidth will not differ among conditions; however, sound frequency may increase with increasing doses of AMPH, similar to the pattern seen with GLU doses.

Methods

Animals & Surgeries

Sixty-eight male Wistar adult rats were used in this study (Charles River, Montreal, QC). Fifty-eight rats underwent stereotaxic surgery at which time they weighed between 190 and 300g. These rats were used for experiments 2 through 5. Ten non-operated rats were used for experiment 1. The animals were kept in pairs before surgeries and placed in single cages after surgeries. The animals were housed in clear plastic cages with a 12:12 hr light/dark cycle with food and water available ad libitum. Approximately 15 minutes prior to the anesthetic, the animals received 0.01 mg/kg of buprenorphine (Rickett & Colman Pharmaceuticals Inc., Hull, England) as an analgesic. Animals were anesthetized with a mixture injection of ketamine hydrochloride (Ayerst Veterinarian Laboratories, Guelf, ON) (40-60 mg/kg, ip) and xylazine hydrochloride (Bayer Inc., Etobicoke, ON) (4-6 mg/kg, ip).[3] An additional half dose of ketamine was administered as a supplementary dose throughout the surgery as needed.

Surgeries were performed in a Kopf stereotaxic apparatus with the incisor bar positioned 3.3 mm below the ear bars. Guide cannulae were bilaterally implanted and aimed 1 mm above the intended injection site in AHPOA using the following coordinates: A: 8.1-8.2 mm from the interaural plane, L: 0.7-0.9 mm from midline, V: 8.2-8.4 mm below the surface of the cortex according to the stereotaxic atlas by Paxinos and Watson (1986). Cannulae were secured to the skull with jeweler screws and methyl methacrylate resin (Perm, Hygenic Corporation of Canada Inc., St. Catharines, ON). Sterile stainless steel plug-pins were used to close the cannulae openings. Animals received 3 ml of warm saline i.p. as fluid replacement immediately following the surgery. Animals were monitored post-surgically and body weight was monitored throughout the testing phase. At the time of testing the rats weighed between 350 and 550 g. All procedures were approved by the Animal Care Committee at Brock University and followed the Canadian

Council on Animal Care guidelines.

Pharmacological Agents and Injection Procedures.

D-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) and sodium L-glutamate (Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% isotonic saline. Saline (Sal) was used as a control vehicle for both AMPH and GLU. AMPH was administered at low (1.5 mg/kg), medium (2.0 mg/kg), and high (2.5 mg/kg) i.p. doses and low (10 μg in 0.3 μl), medium (20 μg in 0.3 μl), and high (40 μg in 0.3 μl) i.c. doses. GLU was administered at a single dose (17 μg in 0.2 μl) for experiments with AMPH and at a higher dose (34 μg in 0.3 μl) for the experiment with haloperidol (HAL). Fu and Brudzynski (1994) reported that a dose of 67.7 μg, but not 34 or 16.6 μg, was effective in increasing the number of 50 kHz calls emitted by rats. However, the lower dose was used to determine if AMPH would increase the effect of GLU at that level. A lower dose was also used to avoid a potential ceiling effect of using both AMPH and GLU together. Haloperidol (Precision Biochemicals Inc., Vancouver, BC) was dissolved in a 1% lactic acid solution and administered at a dose of 2.0 mg/kg, i.p. Lactic acid was used as a control vehicle for HAL.

All i.p. injections were done in a volume of 0.15 - 0.25 ml. The i.c. injections were performed with a CR-700 Hamilton microsyringe in a volume of 0.2 - 0.3 μl unilaterally. The rate of i.c. injections was 10 - 20 nl/s and the injecting cannula was left in place for 10 s before withdrawal. Not more than 8 injections were performed into one brain site.

Experimental Design (see Table 2)

All animals which underwent stereotaxic surgery began the testing phase after a recovery period of 7 - 14 days during which time the animals were weighed and/or handled twice per week. See Table 2 for a breakdown of the conditions in each

experiment.

Experiment 1: Effects of systemic AMPH alone. Ten non-operated rats received a medium dose AMPH, i.p. alone and vehicle injections of equivalent volumes of Sal in a counterbalanced order.

Experiment 2: Effects of systemic AMPH on GLU-induced calls. Twenty-one rats underwent surgical implantation of cannulae into the brain and began the testing. Two did not complete the testing phase due to blocked cannulae such that intracerebral injections could not be performed. AMPH was given at the three doses mentioned above 10 min prior to an intracerebral injection of GLU into the AHPOA. Vehicle injections of equivalent volume of Sal were used as controls for both AMPH (i.p.) and GLU (i.c.).

The following testing conditions were used: i.c. GLU pre-treated with low i.p. AMPH (LoAmph/Glu), i.c. GLU pre-treated with medium i.p. AMPH (MdAmph/Glu), i.c. GLU pre-treated with high i.p. AMPH (HiAmph/Glu), i.c. GLU pre-treated with i.p. Sal (Sal/Glu), i.c. Sal pre-treated with low i.p. AMPH (LoAmph/Sal), i.c. Sal pre-treated with medium i.p. AMPH (MdAmph/Sal), i.c. Sal pre-treated with high i.p. AMPH (HiAmph/Sal), and i.c. Sal pre-treated with i.p. Sal (Sal/Sal) (refer to Table 2).

Nine rats from the first group underwent 8 testing sessions. For sessions 1 and 2, rats received counterbalanced treatments of LoAmph/Glu and Sal/Glu. For sessions 3 and 4, rats received counterbalanced treatments of MdAmph/Glu and Sal/Glu. For sessions 5 and 6 rats received counterbalanced treatments of HiAmph/Glu and Sal/Glu. For sessions 7 and 8 rats received counterbalanced treatments of MdAmph/Sal and Sal/Sal. The rats were no longer used after eight sessions due to possible damaged intracerebral injection site from repeated injections. Therefore, these rats were not tested in the LoAmph/Sal nor the HiAmph/Sal conditions.

Ten additional rats were included to increase the degrees of freedom. Because of

blocked cannulae three rats did not undergo the LoAmph/Sal condition and one rat did not undergo the MdAmph/Sal.

Experiment 3: Effects of intra-AHPOA AMPH on intra-AHPOA GLU-induced calls. Eight rats underwent surgical implantation of cannula and completed the testing phase. For this experiment, AMPH was administered at the medium dose described above into the AHPOA. Vehicle injections of equivalent volume of Sal were used as controls for both AMPH and GLU. Rats were tested in both of the following conditions: i.c. GLU pre-treated with medium i.c. AMPH (MdAmph/Glu) and i.c. GLU pre-treated with i.c. Sal (Sal/Glu), both in a volume of 0.2 µl.

Experiment 4: Effects of higher and lower doses of i.c. AMPH on 50 kHz calls. Ten rats underwent surgery and completed the testing phase. Based on the preliminary results from experiment 3, AMPH was not expected to interact with GLU at the AHPOA to initiate calls, and thus, the effects of AMPH alone into the AHPOA were examined. Intracerebral AMPH was given at higher and lower doses than the previous experiment (as described above), in a volume of 0.2 µl. Rats were tested in each of the following conditions: high i.c.AMPH (HiAmph), low i.c. AMPH (LoAmph), and i.c. Sal (Sal).

Experiment 5: Effects of systemic haloperidol on intra-AHPOA GLU-induced calls. Ten rats from another study and 10 other rats underwent surgery and completed the testing phase. Rats were administered a higher dose of GLU i.c. (34 µg in .3 µl) pre-treated with i.p. HAL (Hal/Glu) and vehicle[4] (Ctrl/Glu) in a counterbalanced order. A higher dose of GLU was used because it was hypothesized that HAL would decrease the number of GLU-induced calls therefore a dose with a stronger effect was used (based on Fu & Brudzynski, 1994) and because a ceiling effect was not expected to occur.

Recording and Analysis of Vocalizations

Immediately following the pre-treatment injection each the rat was placed in the

recording chamber and observed for 10 minutes and the number of vocalizations was recorded. After 10 minutes each rat was removed from the chamber and administered the intracerebral injection and placed back in the chamber for an additional 10 min period of observation and vocalizations were recorded, except for experiment 4 in which only one 10-min period of observation occurred after the i.c. injections. After the testing session was complete rats were returned to their home cages.

The recording chamber consisted of a padded, echo-free recording cage (25 cm wide X 18 cm deep X 18 cm high) housed in a larger sound-resistant, ventilated, and temperature-controlled cubicle (BRS/LVE Tech Serv, Beltsville, MD). Vocalizations were recorded through an ultrasonic microphone model SMI (Ultra Sound Advice, London, England, working range 10-180 kHz) which was mounted in the centre of the side wall and connected to the S200 bat detector (QMC Instruments Ltd., London, England). Signals from the broadband output of the bat detector with the frequency division ratio 1/16 were stored on audio tape for later sonographic analysis of single call duration (in ms), sound frequency (in kHz), and bandwidth (in kHz). Stored vocalizations were subsequently analyzed by a sonograph (DSP Sona-Graph, Kay Elemetrics Corp., Pine Brook, NJ) to obtain sonograms and power spectra from single calls. The first 20 vocalization from each responding rat from each treatment condition were analyzed and a mean for each acoustic parameter was taken. The means were used as single values for each parameter and analyzed statistically. When the rat vocalized less than 20 times, the mean was taken from the number of the calls that were emitted. Means were not taken for rats with fewer than 4 calls. Response latency (time in seconds to the first vocalization emitted after the intracerebral injection) was recorded and response duration (length of time in minutes from the onset of first vocalization to the last vocalization after which the rat did not vocalize for two minutes) was determined from the recorded vocalizations.

Histological Verification

After completion of experiments, animals were sacrificed by an overdose of sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Hamilton, ON), injected with 0.1 µl of 2:1 diluted suspension of India ink into the cannulae to localize injection sites, and immediately transcardially perfused with a 10% solution of formalin. The brains were fixed in 10% formalin and were sectioned, no earlier than 48 hrs after the perfusion, on a freezing microtome (Hacker Freezing Microtome) for 60 - 70 µm preparations, air-dried, delipidized, and stained with an H_20 solution of thionine stain (Gure, 1953). Histological preparations were analyzed under microscope and maps of injection sites were composed according to the stereotaxic atlas by Paxinos and Watson (1997).

Data Analysis

Repeated measures ANOVA or dependent t-tests were used where appropriate to test the hypothesis that AMPH increases the effect of GLU-induced 50 kHz vocalizations by increasing the number of 50 kHz calls emitted by rats. Greenhouse-Geisser correction for non-sphericity was used when necessary. Polynomial contrasts were used to test any dose-response effects. All analyses were done using SPSS computer software.

Repeated measures ANOVA and dependent t-tests were also used to test for differences in acoustical parameters of across conditions. Independent t-tests were used when appropriate for testing the differences between groups. Post hoc tests were used when appropriate.

Results

Histological Verification of Intracerebral Injection Sites

Localization of the intracerebral injection sites is illustrated in Figure 2a & b. Injection sites are labeled as circles with shaded circles indicating sites in which the rats responded to the i.c. injections of GLU (figure 2a) and AMPH (figure 2b) and empty circles indicating sites from which the rat did not respond to the i.c. injections. All GLU injections were localized from 7.70 to 9.48 mm anterior to the interaural plane and all AMPH injections were localized from 7.70 to 9.20 mm. The majority of the GLU injections were in the 8.70 and 8.74 planes and the majority of the AMPH injections were between 8.20 and 8.74 mm anterior to the interaural line. GLU (pretreated with Sal) was injected into 53 brain sites, 35 of which resulted in 50 kHz calls being emitted. AMPH (only) was injected into 17 brain sites, 10 of which resulted in 50 kHz calls being emitted.

Behavioral Analyses

Initial analyses of the raw data revealed that the standard deviations differed by more than four times across some conditions. Because the data were in the form of counts (i.e. number of calls) the mean is often proportional to the variance and therefore square-root transformations of the data are recommended (Howell, 1992). For example, in experiment 2 there was a high, significant correlation between the condition means and their respective variances ($r = .918$, $p < .05$). The transformed values were used for all analyses of number of calls in each experiment. A constant value of 1 was added to all raw data prior to transforming the scores because of several cases in which there were no calls (i.e. a value of 0). The transformation procedure reduced the differences in standard deviations to a difference below 4 times.

Experiment 1: Effects of systemic AMPH alone on 50 kHz calls. Of the ten rats in this experiment, all but 2 showed a more than 10-fold increase in the number of calls emitted after the systemic AMPH injection (M = 835.1, SD = 718.80) compared to the effects after the Sal injection (M = 15.10, SD = 19.62) within the 10-minute observation time. One of the two rats which did not show this pattern did not call at all in either condition while the other called only 9 times after Sal and not at all after AMPH. A dependent t-test was performed on the transformed number of calls data and the results supported the descriptive data, t (9) = 3.561, p = .006. These results show that systemic AMPH significantly increases the number of 50 kHz calls emitted by rats tested individually (Figure 3).

The effects of AMPH were also examined with respect to the influence of AMPH on response latency (time in seconds from the injection to the first vocalization) and response duration (length of time in minutes from the onset of the first vocalization to the last vocalization). The only significant effect that emerged was that rats called for a longer period of time within the 10 min default time after AMPH as compared to Sal, t (9) = 4.247, p = .002. AMPH, however, did not influence response latency, t (9) = .947, n.s. (Figure 3).

These results suggest that AMPH not only increased the number of calls but also increased the duration of calling. These results, along with previous reports in the literature of GLU inducing short, high-frequency ultrasonic vocalizations, justify experiment 2 which examines the joint role of AMPH and GLU in initiating these calls.

Experiment 2: Effects of systemic AMPH on intra-AHPOA GLU-induced calls. A total of 18 rats was used to test the hypothesis that systemic AMPH would increase the number of intra-AHPOA GLU-induced calls. Because the 18 rats consist of two groups which underwent slightly different testing conditions (see methods section) an initial 2 X 6

mixed ANOVA was conducted with group as the between-subject factor and treatment condition as the within-subject factor (Sal/Sal, Sal/Glu, LoAmph/Glu, MdAmph/Sal, MdAmph/Glu, HiAmph/Glu) to determine whether the difference in testing condition required that the data from the groups be analyzed separately. This analysis yielded a marginally significant effect of testing group, F (1,16) = 4.516, p = .050. However, the group by condition interaction was not significant, F (5, 80) = 2.161, p = .104, and thus the group effect was due to an overall increase in the number of calls emitted by the second testing group across conditions. Therefore, the two testing conditions were collapsed and analyses were conducted on all 18 rats.

Another preliminary analysis was performed because the testing phase of the first testing group was such that the first three treatment conditions were counterbalanced with the same control condition of Sal/Glu (see methods section), and hence, the examination of a difference among the three resulting Sal/Glu conditions was of interest. A repeated measures ANOVA with time as the within-subject factor revealed no significant differences among Sal/Glu control conditions, F (2, 16) = 1.109, p = .347. Thus, the first Sal/Glu condition for all rats of the first group was used in the following analyses.

A one-way repeated measures ANOVA was conducted on the six drug conditions with controls (Sal/Sal, Sal/Glu, MdAmph/Sal) and AMPH-GLU conditions (LoAmph/Glu, MdAmph/Glu, HiAmph/Glu). The analysis revealed a significant effect of treatment condition on the number of calls, $F(5,85) = 16.312$, $p < .001$. Post hoc tests using Bonferroni's t-test (adjusted for multiple comparisons) revealed that Sal/Sal and Sal/Glu did not differ from each other ($p = .247$) but there were fewer ($p \leq .019$) calls emitted in both of those two conditions compared each of the four AMPH conditions (LoAmph/Glu, MdAmph/Sal, MdAmph/Glu, HiAmph/Glu), which themselves did not differ, $p \geq .247$ (Figure 4 a).

An AMPH (AMPH vs Sal) by GLU (GLU vs. Sal) repeated measures ANOVA

was also conducted to test for an interaction between the medium dose of AMPH and GLU because it was only possible with all 18 rats for these four conditions. There was a significant main effect of AMPH, F (1, 17) = 40.172, $p < .001$ but no main effect of GLU, F (1, 17) = 1.408, $p = .252$. There was also a significant interaction, F (1, 17) = 5.027, $p = .039$, which qualified the main effect of AMPH showing that when GLU was pretreated with AMPH, the number of calls was reduced slightly compared to AMPH pretreatment of Sal (Figure 4 b).

A 4 (AMPH) X 2 (GLU) repeated measures ANOVA was performed on the six rats which served in all eight conditions to test the interaction between AMPH and GLU. A main effect of AMPH emerged, F (3, 15) = 4.711, $p = .046$, but no main effect of GLU, F (1, 5) = 1.293, $p = .307$, nor an AMPH by GLU interaction, F (3, 15) = 3.191, $p = .109$ emerged. The results of this analysis would suggest that the effect of increased calls is a function of AMPH alone (Figure 4 c), although caution should be exerted because this analysis incorporated the data from only six animals whereas the previous analysis incorporated that of 18 animals.

Although the analysis above indicated that GLU did not induce calls compared to Sal a comparison was done between the 10-min period prior to the intra-AHPOA GLU injection (i.e. after systemic Sal) and the 10-min period after the GLU injection. This comparison was statistically significant, t (20) = 2.357, $p = .029$, indicating that there were more calls emitted after the intra-AHPOA injection of GLU than there were prior to the injection. However, a Time (first 10 min vs. second 10 min observation periods) by Treatment (Sal vs. GLU) repeated measures analysis indicated that the effect was merely due more calls being emitted during the second 10 min observation time (i.e. a main effect of Time, F (1, 18) = 11.062, $p = .004$). There was no main effect of Treatment, F (1, 18) = 2.628, $p = .122$, nor was there an interaction, F (1, 18) = .001, n.s. Thus, the effect of GLU was negligible. The acoustics, response latency, and response duration data are not

available for the pretreatment phases of this experiment and therefore could not be analyzed.

The data were also examined for an effect of AMPH irrespective of GLU administration (see Figure 4d). However, only the rats from the second testing session underwent the low and high doses of systemic AMPH administration with Sal, as opposed to with GLU. Of those ten animals, only six completed all sessions. Thus, with these small degrees of freedom, the dose-dependent relationship only approached significance, $F(3, 15) = 3.649$, $p = .089$, however, a quadratic relationship was observed, $F(1, 5) = 19.056$, $p = .007$, suggesting that the number of calls emitted by rats increases at low doses and decreases with higher doses.

Response latency and response duration were also analyzed to determine if a difference existed in the time before the first call was emitted and the duration of calling after AMPH or Sal injections. A repeated measures one-way ANOVA with the same six levels of drug administration was performed on the response duration data which revealed that response duration was significantly affected by the GLU and AMPH administration, $F(5,85) = 26.516$, $p < .001$. The effect was due to a shorter response duration in the Sal/Sal and Sal/Glu conditions compared to all of the AMPH conditions (Figure 4e), according to Bonferroni's Post Hoc t-test (adjusted for multiple comparisons). All significant p values were equal to or less than .003 and all nonsignificant p values were equal to or greater than .722.

A repeated measures one-way ANOVA was also performed on the response latency and an overall significant difference emerged, $F(5,85) = 4.689$, $p = .009$. The effect appears to be due to a trend for longer latencies in the Sal/Sal and Sal/Glu conditions compared to all AMPH conditions (Figure 4e), which was generally supported by LSD Post Hoc t-tests.

Together, the results of experiment 2 further supported the conclusion that DA has

a significant role in influencing 50 kHz calls. Systemic AMPH significantly increased the number of calls and duration of the response with a trend for shortening the latency to the first call emitted. These results warranted the investigation into the effects of AMPH directly on the local glutamatergic system in the AHPOA in producing 50 kHz calls.

Experiment 3: Effects of intra-AHPOA AMPH (20 μg) on intra-AHPOA GLU-induced calls. Eight rats served in two conditions: Amph/Glu and Sal/Glu. The third experiment was conducted to test the hypothesis that AMPH acts on the glutamatergic transmission directly in the AHPOA. A Time (pre, post) by Pre-treatment (Sal, AMPH) repeated measures ANOVA was conducted on the number of call data. The analysis resulted in a significant main effect of Time, F, (1, 7) = 7.810, p = .027, which shows that administration of GLU increased the number of calls emitted. There was no significant main effect of the Pre-treatment, F (1, 7) = 1.357, p = .282, and no significant interaction, F (1, 7) = .946, n.s. However, because each individual comparison was of interest three dependent t-tests were performed to test: a) AMPH vs. Sal (i.e. comparing the first 10 min period following i.c. AMPH or Sal with each other), b) AMPH Pre-treatment period vs. Amph/Glu treatment (i.e. the first 10 min period following the AMPH pretreatment compared to the second 10 min period following the Glu treatment after the initial AMPH pre-treatment), and c) Amph/Glu vs. Sal/Glu (i.e. GLU pretreated with AMPH vs. GLU pretreatment with Sal).

Surprisingly, the first t-test resulted in a significant effect of rats emitting fewer calls in the AMPH condition than the Sal condition, t (7) = 2.525, p = .040. For the second comparison, there were only marginally fewer calls in the AMPH pretreatment period compared to the Amph/Glu period, t (7) = 2.50, p = .080. However, there was no difference seen between the Amph/Glu condition and the Sal/Glu condition, t (7) = .311, n.s. (see figure 5a for a depiction of the results).

Response latency and response duration were also considered between each pairing (Figure 5b). The results of only one t-test approached significance, t (7) = 2.80, p = .057. Response duration was marginally shortened with AMPH compared to Sal. All other comparisons resulted in p values greater than .121, and hence not significant.

Because AMPH did not seem to interact with GLU to increase the number of 50 kHz calls within the studied area of the AHPOA to produce 50 kHz calls – neither when administered systemically or intracerebrally – experiment 4 was designed to examine the effects of intra-AHPOA AMPH alone on 50 kHz calls. Doses half that, and twice that, used in experiment 3 were chosen for experiment 4 arbitrarily due to a lack of previous dose-response studies of AMPH administration into the AHPOA.

Experiment 4: Effects of higher (40 µg) and lower (10 µg) doses of intra-AHPOA AMPH on 50 kHz calls. A repeated measures ANOVA with higher and lower doses of AMPH with a Sal control was performed on the call data of nine rats. The analysis failed to show an effect of dose on the number of calls, F (2,16) = .415, n.s. However, the trend, albeit not significant, was in the same direction such that fewer calls were emitted after AMPH compared to Sal (M = 8.98 Sal; M = 8.04, LoAmph; M = 6.32, HiAmph, no figure available).

Response latency and response duration were again considered despite the lack of significant effect of AMPH on the number of calls. A repeated measures ANOVA across the two doses of AMPH and the Sal control failed to show an effect of AMPH for either response latency or response duration, F (2,16) = .044, n.s. and F (2,16) = 1.587, n.s, respectively. A graph of the means for latency (sal: 2.83, SD = 4.30; Lo: 2.58, SD = 4.24; Hi: 2.25, SD = 4.39) and duration (sal: 5.56, SD = 3.84; Lo: 3.73, SD = 4.06; Hi: 3.11, SD = 3.26) is therefore not presented.

The results from the first four experiments together suggest that although DA

plays a significant role in increasing 50 kHz calls, the effect does not appear to be mediated directly at the AHPOA as it is with GLU. To further study the possible GLU-DA relationship experiment 5 was designed to test the effects of antagonizing DA with a systemic injection of HAL.

Experiment 5: Effects of systemic haloperidol on intra-AHPOA GLU-induced calls. It was hypothesized that antagonizing DA receptors with HAL would suppress the number of GLU-induced 50 kHz calls. HAL was administered at a dose of 2.0 mg/kg in 20 rats 10 min prior to an intracerebral injection of GLU into the AHPOA. The dose of GLU was increased to 34 μg in 0.3 μl because GLU did not appear to robustly increase the number of 50 kHz calls at the lower dose used in the previous experiments. Because the interest was in suppressing calls, and not in increasing them, the use of a higher dose was justified.

Some preliminary analyses were conducted to examine the effect of the control treatments in which an improper vehicle of Sal was used rather than lactic acid for the first 10 rats tested in this experiment.[3] The number of calls emitted by the rats pretreated with Sal or lactic acid prior to GLU were compared. A t-test indicated that the two control groups did not differ significantly, t (18) = .682, n.s., and thus collapsing the two groups into one was warranted.

Also, a dependent t-test was conducted to determine if an effect of GLU on the number of calls would emerge, thus providing the basis for attempting to reduce the effect with a DA antagonist. The results indicated that rats emitted fewer calls during the 10-min period prior to the intra-AHPOA GLU injection (i.e. after the systemic control injection) than during the 10-min period following the intra-AHPOA GLU treatment, t (19) = 3.150, p = .005.

Thus, with these premises confirmed, a t-test was performed to determine whether

systemic haloperidol suppressed GLU-induced 50 kHz calls. The claim was supported, t (19) = 2.256, p = .036 (Figure 6) showing that the effects of systemic HAL antagonized the call-inducing effect of GLU.

T-tests were performed on the response latency and response duration data to examine the effect of haloperidol on these parameters. In both cases, haloperidol had a significant effect; response latency was lengthened, t (19) = 3.787, p = .001, and response duration was shortened, t (19) = -3.879, p = .001, with haloperidol administration (Figure 6).

Sonographic Analyses

Sonographic analyses were not possible for all experiments because many rats in the control condition and the HAL condition did not emit any calls and therefore could not be compared to calls emitted after AMPH and/or GLU administration. Table 3 presents the range for peak sound frequency, single call duration, and bandwidth for each condition and of each experiment. All call means were within the 35 - 70 kHz range of peak frequency commonly reported in the literature as typical '50 kHz' calls.

In experiment 1 only five rats emitted calls across both AMPH and Sal conditions allowing only a limited degrees of freedom for the t-test. The t-tests did fail to reveal any significant difference between conditions on any acoustic parameter. The means are presented in Table 3.

In experiment 2 data from only 10 rats were submitted to the repeated measures ANOVA because the other 10 rats did not emit calls in one or more of the conditions. None of the parameters differed significantly among conditions: sound frequency, F (5,45) = 1.236, n.s. single call duration, F (5,15) = 1.144, n.s.; and bandwidth, F (5,45) = .785, n.s. Call frequency means ranged from 52.6 kHz to 56.2 kHz, all within the range of 35 - 70 kHz, call duration means ranged from 33.2 ms to 40.26 ms, and bandwidth means

ranged from 7.0 kHz to 8.2 kHz (see Table 3).

For experiment 3, t-tests were conducted between AMPH and Sal pre-treatments, between AMPH alone and GLU pre-treated with AMPH, and between GLU pre-treated with AMPH and GLU pre-treated with Sal. Significance was not reached in any pairing, however, the degrees of freedom in each pairing were less than 5 because not all animals emitted calls in each condition. Thus, too much weight cannot be placed upon this lack of significance. A similar problem arose when comparing the acoustic parameters in experiment 4 wherein only 3 rats called throughout all the conditions thus limiting the degrees of freedom.

In experiment 5, again, only 3 rats emitted calls across both haloperidol and Sal conditions leaving a degrees of freedom of 2. However, a significant effect did emerge for call duration such that calls emitted after HAL administration were longer than those emitted after Sal, t (2) = 5.652, p = .030. Again, caution should be exerted due to limited degrees of freedom.

Discussion

The general hypothesis was that the dopaminergic system plays a mediating role in GLU-induced 50 kHz vocalizations in adult rats. Although the GLU-DA relationship did not emerge with systemic injections of AMPH, it did emerge when DA receptors were blocked with HAL and GLU-induced calls were inhibited. In addition, a strong relationship between DA and 50 kHz calls, irrespective of GLU, was observed with systemic AMPH administration. The results strongly support a role for both AMPH and GLU in the initiation of 50 kHz calls.

Number of calls

Experiment 1

Prior to examining the DA-GLU relationship, the role of DA in the production of this type of call was investigated using systemic AMPH, a dopaminergic agonist, which effectively increased the number of calls (Figure 3). The present results, along with those presented by Burgdorf et al. (2000) and Knutson et al. (1997, 1998, 1999) strongly support the role of DA in the production of 50 kHz calls. Together, these results document that dopaminergic agonists can initiate and increase the number of calls emitted by adult rats. The present results, along with previous reports of 50 kHz calls being induced by AMPH injections to the N.Acc. (Burgdorf & Panksepp, 1999), 50 kHz calls occurring in anticipation of electrical brain stimulation to the VTA and lateral hypothalamus (Burgdorf et al., 2000), and 50 kHz calls representing conditioned reward (Knutson, et al., 1999) provide a strong argument for the role of dopaminergic reward system in the initiation of 50 kHz calls.

Experiment 2

The second experiment was an attempt to determine whether DA had a mediating role in the GLU-induced 50 kHz calls. The results of experiment 2 showed that intra-

AHPOA GLU alone, at a low dose (17 μg), did not have a robust effect in increasing the number of calls emitted by rats because the effect was only significant when the 10 min period prior to the GLU injection was compared to the 10 min period post-injection and not when comparing the results of the Sal pretreatment/Sal treatment condition to the Sal pretreatment/GLU treatment condition. GLU has been previously shown to exert dose-dependent effects; however, the dose used in the present experiment was not shown to be effective albeit higher doses were (Fu & Brudzynski, 1994).

AMPH, however, did exhibit a robust effect on the number of calls emitted by rats at each of the three doses tested (Figure 4a). The effect of GLU pretreated with AMPH was only fully examined with the medium dose (2.0 m/kg) of AMPH because not enough data were available to examine the effect at the other doses in comparison to the control conditions (i.e. LoAmph/Sal and HiAmph/Sal) (Figure 4c). An attempt was made with the data of the few rats that were available; however, the same effect did not emerge when all three doses with the three Sal control conditions were analyzed (Figure 4d). Unfortunately, the results are limited to interpreting the effect with the medium dose, and further analysis should be done on the other doses of AMPH to more clearly portray the relationship of intra-AHPOA GLU and systemic DA manipulations.

Also, as inferred from the data of experiment 2, a dose-response relationship of AMPH exists such that at the lower doses (1.5 mg/kg and 2.0 mg/kg) the number of calls increased with the AMPH dose whereas with the highest dose (2.5 mg/kg) the number of calls emitted decreased (Figure 4d). This important quadratic relationship may have implications in understanding the effect of intra-AHPOA GLU in reducing the number of calls when administered in conjunction with higher doses of AMPH. One possible reason for these results may be that an optimal level of DA transmission exists and when transmission increases past or decreases below such a level fewer calls are emitted. Thus, an important extension to the present results would be to examine the dose-response, in

particular, with AMPH doses exceeding 2.5 mg/kg.

Experiment 3 & 4

The third and fourth experiments were conducted to consider mediating role of DA and GLU at the AHPOA site for 50 kHz calls (see Figure 5a for experiment 3; no figure for experiment 4). The results were ambiguous and counter to the predictions. First, intra-AHPOA AMPH induced fewer calls than the control injection of Sal, which was opposite to what was predicted. Also there were marginally fewer calls in the 10-min pretreatment period with AMPH compared to during the 10-min treatment period of GLU (i.e. subsequent to AMPH pretreatment), suggesting that GLU partially compensated for the fewer calls seen with AMPH alone. The expected effect of GLU was also significant when comparing the number of calls from the 10-min Sal period (i.e. pre-GLU) with the calls during the 10-min GLU treatment period. However, there was no significant difference in the number of calls emitted when GLU was pretreated with AMPH compared to a pretreatment with Sal. Therefore, it appears from experiment 3 that at the AHPOA site AMPH may reduce the number of calls emitted by rats at the dose level studied here but that GLU (also into the AHPOA) can almost completely compensate for the reduction when administered shortly after AMPH.

The results of experiment 4 further complicate the results of intra-AHPOA AMPH, which revealed that the number of calls was reduced, because a significant dose effect did not emerge overall, nor as any polynomial trend. Although the means decreased with increasing AMPH doses, including the control condition, the difference was not significant. Furthermore, no information regarding the GLU-DA relationship at the AHPOA exists because GLU was not used in experiment 4.

As alluded to above, the relationship between GLU and DA is complex in other behaviors, so perhaps it is not surprising that a complicated relationship exists for 50 kHz calls as well. As mentioned earlier, some authors argue that locomotor activity is only

blocked by GLU receptor sub-type antagonists AMPA and not NMDA (Burns et al, 1994; Freed & Cannon-Spoor, 1990) suggesting a receptor-mediated specificity. Also, some GLU agonists, such as NMDA, have been shown to block other DA-dependent behaviors, such as conditioned responding (Burns et al., 1994) and sensitization (Wolf & Khansa, 1991). A clear understanding of the relationship between DA and GLU in mediating other behaviors does not exist and it may be naive to assume that the relationship in mediating 50 kHz calls is any more intelligible. The results of experiment 3 confound both lines of thinking and the action of DA at the AHPOA may need to be further explored.

Experiment 5

The last experiment was conducted to show that antagonizing DA receptors, and effectively decreasing DA action through systemic injection of HAL prior to GLU, would suppress GLU-induced calls. The results of experiment 5 provide strong, further evidence of the role of DA in the production of 50 kHz calls. When DA receptors are blocked, GLU-induced calls are significantly reduced. This result provides the first important indication that DA is necessary for initiation of 50 kHz calls by GLU. A similar conclusion was reached regarding locomotor activity (Wu, M., Brudzynski, S. M., & Mogenson, G. J., 1993). NMDA and AMPA (GLU agonists) injections into destroyed DA terminals of the N.Acc. significantly reduced NMDA- and AMPA-induced locomotor activity. Thus, the authors concluded that although both DA and GLU are necessary for the increased locomotor behavior, GLU-induced response is DA-dependent.

Response Latency and Duration

A relationship between the response parameters and treatment conditions exists that parallels that of the number of calls. In both experiment 1 and 2 response duration was lengthened with systemic AMPH administration and the number of calls increased whereas response duration was shortened in experiment 3 and the number of calls

decreased. Response duration was unaffected by intra-AHPOA AMPH in experiment 4 and similarly was the number of calls. Furthermore, in experiment 5, where the number of calls decreased with systemic HAL administration, so did response duration.

Latency, in comparison, was less affected by DA manipulations. In experiment 1, latencies were not affected and in experiment 2 were only slightly influenced by AMPH administration such that rats took longer to emit the first call after Sal/Sal and Sal/Glu conditions than after conditions with systemic AMPH. Intra-AHPOA AMPH, with (experiment 3) and without (experiment 4) GLU, again did not influence response latency. However, with systemic haloperidol administration, it took rats much longer to emit their first call than with the control injection.

Together, the response parameters indicate that, not surprisingly, when the number of calls increases, such as with AMPH, response duration is lengthened and latency is shortened and when the number of calls decreases, such as with HAL, duration is shortened and latency is lengthened. This relationship further supports the conclusion that the calls emitted are a result of the DA manipulations because it was not only the number of calls that was influence by DA transmission, but also response latency and duration. The response latency, however, seems to be less useful in assessment of quantitative differences.

Acoustic Parameters

There was only one experiment in which significant results of acoustic characteristics emerged. The calls emitted by rats when GLU was pretreated with HAL, compared to pretreated with Sal, the calls were longer. However, it should be re-emphasized that there were only two degrees of freedom associated the analysis and therefore strong inferences from the data should not be made. In the literature, only call frequency has been reported to change with changes in intra-AHPOA dose of GLU (Fu &

Brudzynski, 1994). Call frequency increased as intra-AHPOA GLU doses increased. Thus, the change in call duration between HAL and control conditions may be the result of individual differences of only three rats and not a meaningful or reliable difference. However, because the methodological problem with the present acoustical analysis lies in the fact that rats tend not to call during the control condition, or call very little, it might be also of interest to compare AMPH-induced or HAL-inhibited 50 kHz calls with calls induced naturally, such as with anticipation of food reward which Burgdorf et al. (2000) found to elicit 50 kHz. Thus, an acoustical comparison could be done which could provide information on whether the calls induced pharmacologically differ from those induced naturally.

Alternative Hypotheses

Although the increase in the number of calls emitted after AMPH, and a decrease with HAL, pretreatments of GLU were considered an action of AMPH on the "vocalization system" an alternative hypothesis is that DA agonists and antagonists, increase and decrease, respectively, locomotor activity and that 50 kHz call rate is a direct function of arousal and/or locomotor activity. Although in the present set of experiments locomotor activity was not measured as a covariate of the number of calls, locomotor activity has been previously considered and concluded not to be a sole contributor to initiation of 50 kHz calls (Burgdorf et al., 2000; Knutson et al., 1998). In Burgdorf et al. locomotor activity and 50 kHz calls were both recorded in 5 second bins leading up to electrical brain stimulation. The authors reported that both the number of 50 kHz calls and locomotor activity systematically increased, however, the effect of electrical brain stimulation on the number of 50 kHz calls remained significant even after removing the effects due to locomotor activity. The same authors also reported earlier that juvenile rats emitted more 50 kHz calls on Day-2, compared to Day-1, of a two-day testing paradigm

despite activity being significantly higher on day two for one group and significantly lower on day two for the other group (Knutson et al., 1998).

Dissociating vocalization from activity can prove to be a difficult task because the dopaminergic system is involved in initiating locomotor activity (see Feldman et al., 1997) and also mediates the reward attached to behaviors and stimuli (see Engel et al., 1987). Although to separate the two behaviors may be feasible, it may not be completely justified or correct to do so. The two behaviors may be functionally linked through the dopaminergic system and to remove the effects of one may remove part of the effects of the other which necessarily requires the same physiological component to initiate either behavior. This would explain why locomotor patterns are similar to that of 50 kHz leading up to electrical brain stimulation (Burgdorf et al., 2000).

Another alternative explanation for the observed results is that they are a function of, or confounded by, reward conditioning of the testing situation. Although reward conditioning will potentially be a factor in any experiment with repeated administration of rewarding stimuli, such as AMPH, it is unlikely to account for the results of the present study. First, in experiment 2, where half the rats used followed counterbalanced treatments and half did not, only an overall group effect emerged showing that more calls were emitted by rats in the second group. The lack of group by condition interaction suggests that, although the second set of rats called more, no condition was uniquely affected by the overall increase. Had the conditions differed as a function of group, the possibility of an influence of order of treatments could not be ruled out. However, with the group effect, it can been assumed that any conditioning that occurred added a constant to each session and hence conditioning would not be of major concern.

Also, if conditioning had influenced the present results, an effect of testing time would have been apparent across the three Sal/Glu conditions (time 1, 2, & 3) of half the rats in experiment 2; however, this was not the case. For the rats in experiment 2, a

Sal/Glu condition was counterbalanced with increasing doses of AMPH for the first three weeks of testing. Thus, had conditioning occurred to a large extent, more calls would have been emitted in the third Sal/Glu condition compared to the first or second condition, and this was not the case.

Limitations

There are some limitations of this study which deserve acknowledgment. A major limitation is in regards to the acoustic analyses. Although the acoustic parameters served the purpose of characterizing the calls as short, 50 kHz, they were not useful in testing for differences among conditions, as was intended. This limitation is a shortfall of the testing paradigm which did not promote 50 kHz calls in the absence of rewarding and appetitive situations or stimuli, as in some of the control conditions. However, based on the present results of clear increases in the number of calls with AMPH compared to control conditions, it would be advantageous to provide a rewarding or appetitive environment for animals under both control and AMPH conditions. Situations could include those conditions which have been reported to induce natural calling such as playing and anticipation of food (Knutson et al, 1998). AMPH and Sal controls could be administered in a rewarding environment to observe the effect over and above natural reward. Prior to the present study, one would have been cautious to test in such situations because the number of calls emitted during natural situations and pharmacological situation may not differ, and thus, no difference would be observed. However, based on the present results, it is clear that the number of calls emitted after AMPH could greatly supersede those naturally induced because of the sheer magnitude of AMPH-induced 50 kHz calls.

Another limitation comes from a limited spread of substances injected into the brain (see Myers, 1974). For example, one microliter droplet of fluid will occupy an area of only 1.1 millimeter in diameter and diffuse not more than 2 mm away. The volume

used in the present set of experiments were 0.2 - 0.3 µl and thus the spread is rather limited. Electrophysiological results indicate that carbachol, in a volume of 0.2 µl, would affect neuronal firing within a region of 1.2 mm in diameter (Brudzynski et al., 1991). Although such a volume into HPOA was effective for other agents including carbachol – and in the present study GLU – AMPH may require a larger volume in order to diffuse enough and influence the critical number of neurons to achieve an effect. Moreover, the vocalization area is not limited to the AHPOA, as mentioned above, and the doses and volumes of AMPH used may not suffice in stimulating enough of the vocalization system to induce 50 kHz calls. Because the dose of AMPH used in these experiments were arbitrarily chosen due to the lack of previous dose-response studies, the doses could have been much less than that needed to stimulate the area to induce 50 kHz calls, and a similar argument could be said for the volumes used. Injections of AMPH to larger areas of the HPOA and in larger volumes could show an effect as robust as that which is seen with systemic administration of AMPH.

Conclusion

The results of this study strongly demonstrate that AMPH and GLU both have mediating roles in producing 50 kHz calls in adult rats. Although GLU is known to have its 50 kHz call-inducing effect at the AHPOA, the primary site of action for AMPH is not the AHPOA. Because others have reported a similar effect at the VTA (Burgdorf et al., 2000) and at the N.Acc (Burgdorf & Panksepp, 1999) it may be that glutamatergic neurons in the AHPOA receives projection from the VTA or that glutamatergic neurons facilitate the release of DA in the reward system. The projections from the VTA do pass through the hypothalamic portion of the MFB to ascend to the N.Acc and cause the release of DA (Feldman et al., 1997; Ungerstedt, 1971). If the AHPOA and the reward system both make up a "50 kHz vocalization strip", it could account for why stimulating

the AHPOA with GLU or electrically stimulating the VTA or chemically stimulating the N.Acc. with DA could each cause an increase in the number of 50 kHz calls, calls which may then reflect a state of reward. Although the present study did not directly assess the appetitive nature of the 50 kHz calls, it supports a dopaminergic role in inducing 50 kHz calls and together with those studies which show that 50 kHz calls are emitted during period of reward and/or reward anticipation (Burgdorf et al., 2000; Knutson, et al. 1999, 1998) the appetitive nature is strongly supported. Furthermore, this study provides the first indication that DA mediates GLU-induced 50 kHz calls which was evident only when DA receptors were antagonized with systemic HAL but not when transmission was increased with AMPH.

Footnotes

1. The striatum consists of 3 subdivisions: the caudate nucleus, putamen. and the ventral striatum (which includes the nucleus accumbens), although often the caudate-putamen is often synonymous with striatum. The striatum, globus pallidus (a.k.a. the pallidum), and substantia nigra together make up structures of the basal ganglia. See Kandel, Swartz, Jessell, 2000, 855-856.

2. Glutamate receptor sub-types are named after their primary agonists. Therefore, the receptor sub-type NMDA is agonized most strongly by the pharmacological agent NMDA. Similarly, AMPA is most strongly agonized by AMPA.

3. At the beginning of the study animals were anesthetized with 60 mg/kg of Ketamine and 4 mg/kg of Xylazine separately but the A.C.C protocol was changed and thus after the first surgery group (those animals from experiment 2) animals were anesthetized with 40 mg/kg or Ketamine and 6 mg/kg of Xylazine as a mixture.

4. Ten rats received a proper lactic acid control while 10 rats received an improper Sal control. The two groups did not differ significantly, $t(18) = -0.063$, $p > .1$, as a result of separate controls and, thus, were collapsed into one group.

References

Antoniou, K., Kafetzopoulos, E., Papadopoulon-Daifoti, Z., Hyphantis, T., & Marselos. (1998). d-amphetamine, cocaine, and caffeine: a comparative study of acute effects on locomotor activity and behavioral patterns in rats. Neuroscience and Biobehavioral Reviews, 23, 189-196.

Bartoszyk, G. D. (1998). Anxiolytic effects of DA receptor ligands: I. Involvement of DA autoreceptors. Life Science, 62, 649-663.

Bell, R. W. (1974). Ultrasounds in small rodents: Arousal-produced or arousal-producing. Developmental Psychobiology, 7, 39-42.

Blanchard, R. J., Blanchard, D. C., Agullana, R., & Weiss, S. M. (1991). Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow system. Pharmacology Biochemistry and Behavior, 44, 313-319.

Blanchard, R. J., Yudko, E. B., Blanchard, D. C., & Taukulis, H. K. (1993). High-frequency (35-70) kHz ultrasonic vocalizations in rats confronted with anesthetized conspecifics: Effects of Gepirone, Ethanol, and Diazepam. Pharmacology , Biochemistry, and Behavior, 44, 313-319.

Blumberg, M. S. (1992). Rodent ultrasonic short calls: locomotion, biomechanics, and communications. Journal of Comparative Psychology, 106, 360-365.

Blumberg, M. S. & Alberts, J. R. (1991). On the significance of similarities between ultrasonic vocalizations of infant and adult rats. Neuroscience & Biobehavioral Reiviews, 15, 383-390.

Blumberg, M. A., Sokoloff, G., Kirby, R. F., & Kent, K. J. (2000). Distress vocalizations in infant rats: What's all the fuss about? Psychological Science, 11, 78-81

Bozarth, M. (1987). Ventral tegmental reward system. In J. Engel, L. Oreland., D. H. Ingvar, B. Pernow, S. Rossner, L. A. Pellborn (Eds.), Brain Reward Systems and Abuse (pp. 1-17). New York, NY: Raven Press Books, Ltd.

Brudzynski, S. M. (1994). Ultrasonic vocalization induced by intracerebral carbachol in rats: localization and a dose-response study. Behavioural Brain Research, 63, 133-143

Brudzynski, S. M. (1998). Abstracts of the International Behavioral Neuroscience Society.

Brudzynski, S. M. & Barnabi, F. (1996). Contributions of the ascending cholinergic pathways in the production of ultrasonic vocalization in the rat. Behavioural Brain Research, 80, 145-152.

Brudzynski, S. M., Bihari, F., Ociepa, D., & Fu, X. (1993). Analysis of 22 kHz ultrasonic vocalizations in laboratory rats: Long and short calls. Physiology & Behavior, 54, 215-221.

Brudzynski, S. M. & Chiu, E. M. C. (1995). Behavioral responses of laboratory rats to playback of 22 kHz ultrasonic calls. Physiology & Behavior, 57, 1039-1044.

Brudzynski, S. M. & Eckersdorf, B. (1984). Inhibition of locomotor activity during cholinergically-induced emotional-aversive response in the cat. Behavioral Brain Research, 14, 247-253.

Brudzynski, S. M., Kadishevitz, L., & Fu, XW. (1998). Mesolimbic component of the ascending cholingergic pathways: electrophysiological-pharmacological study. Journal of Neurophysiology, 79, 1675-86.

Brudzynski, S. M., McLauchlan, R. S., & Girvin, J. P. (1989). Cholinergically mediated reduction of locomotor activity from the basal forebrain of the rat. Experimental Neurology, 105, 197-205.

Brudzynski, S. M. & Mogensen, G. J. (1985). Association of the mesencephalic locomotor region with locomotor activity induced by injections of amphetamine into the nucleus accumbens. Brain Research, 334, 77-84.

Brudzynski, S. M. & Ociepa, D. (1992). Ultrasonic vocalization of laboratory rats ub response to handling and touch. Physiology & Behavior, 52, 655-660.

Brudzynski, S. M., Ociepa, D., & Bihari, F. (1991). Comparison between cholinergically and naturally induced ultrasonic vocalization in the rat. Journal of Psychiatric Neuroscience, 16, 221-226.

Burgdorf, J. & Panksepp, J. (1999). Evidence that rat ultrasonic calls can index both positive and negative affective states. Proceedings from the Society for Neuroscience Annual Meeting, 25, 875.

Burgdorf, J., Knutson, B., & Panksepp, J. (2000). Anticipation of rewarding electrical brain stimulation evokes ultrasonic vocalizations in rats. Behavioral Neuroscience, 114, 320-327.

Burns, L. H., Everitt, B. J., Kelley, A. E., & Robbins, T. W. (1994). Glutamate-dopamine interactions in the ventral striatum: role in locomotor activity and responding with conditioned reinforcement. Psychopharmacology, 115, 516-528.

Cagiano, R., Sales, G. D., Renna, G., Racagni, G., & Cuomo, V. (1986). Ultrasonic vocalization in rat pups: Effects of early postnatal exposure to haloperidol. Life Sciences, 38, 1417-1423.

Corbett, D. (1989). Possible abuse potential of the NMDA antagonist MK-801. Behavioral Brain Research, 34, 239-246.

Corrigan, J. G. & Flannelly, K. J. (1979). Ultrasonic vocalizations or the male rat. Physiological Behavior, 22, 483-489.

Cotman, C. W. & Monoghan, D. T. (1987). Chemistry and anantomy of excitatory amino acid systems. In H. Y. Meltzer (Eds.), Psychopharmacology: The third generation of Progress. (pp. 197-210). New York, NY: Raven Press.

Drinkenburg, W. H., Keith, A. B., Sahgal, A., & Andrews, J. S. (1999). Haloperidol-induced within-session response decrement patterns and catalepsy in rats: behavioural dissociation. Behavioral Pharmacology, 10, 105-111.

Engel, J., Oreland, L., Ingvar, D. H., Pernow, B., Rossner, S., & Pellborn, L. A. (Eds.). (1987). Brain Reward Systems and Abuse. New York, NY: Raven Press Books, Ltd.

Ellinwood, E. H. Jr. & Balster, R. L. (1974). Rating the behavioral effects of amphetamine. European Journal of Pharmacology, 28, 35-41.

Feed, W. J. & Cannon-Spoor, H. E. (1990). A possible role of AA2 excitatory amino acid receptors in the expression of stimulant drug effects. Psychopharmacology, 101, 456-464.

Feldman, R. S., Meyer, J. S., & Quenzer, L. F. (1997). Principles of Neuropsychopharmacology. Sunderland, MA: Sinauer Associates, Inc.

Fibiger, H. C. & Phillips, A. G. (1987). In J. Engel, L. Oreland., D. H. Ingvar, B. Pernow, S. Rossner, L. A. Pellborn (Eds.), Brain Reward Systems and Abuse (pp. 61-74). New York, NY: Raven Press Books, Ltd.

Fu, X. & Brudzynski, S. M. (1994). High-frequency ultrasonic vocalization induced by intracerebral glutamate in rats. Pharmacology Biochemistry and Behavior, 49, 835-841.

Gurr, E. (1953). The Practical Manual of Medical and Biological Staining Techniques. Interscience Publishers Inc.: New York.

Herber, L. J. & Rose, I. C. (1989). The effect of MK-801 and other antagonists of NMDA-type glutamate receptors on brain-stimulation reward. Psychopharmacology, 99, 87-90.

Hoffman, D. C. (1994). The noncompetitive NMDA antagonist MK-801 fails to block amphetamine-induced place conditioning in rats. Pharmacology, Biochemistry, and Behavior, 47, 907-912.

Howell, D. C. (1992). Statistical Methods for Psychology. (3rd ed.). Belmont, CA: PWS-KENT Publishing Co.

Jones, G. H., Marsden, C. A., & Robbins, T. W. (1990). Increased sensitivity to amphetamine and reward-related stimuli following social isolation in rats: Possible disruption of dopamine-dependent mechanisms of the nucleus accumbens. Psycholopharmacology, 102, 364-372.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (2000). Principles of Neural Science (4th Ed.). New York: McGraw-Hill.

Karler, R. & Calder, L. D. (1992). Excitatory amino acids and the actions of cocaine. Brain Research, 582, 143-146.

Karler, R., Calder, L. D., Thain, L. M., & J. B. Bedingfield. (1995). The dopaminergic, glutamatergic, GABAergic bases for the action of amphetamine and cocaine. Brain Research, 671, 100-104.

Kelley, A. E. & Throne, L. C. (1992). NMDA receptors mediate the behavioral effects of amphetamine infused into the nucleus accumbens. Brain Research Bulletin, 29, 247-254.

Knutson, B., Burgdorf, J., & Panksepp, J. (1998). Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. Journal of Comparative Psychology, 112, 65-73.

Knuston, B., Burgdorf, J., & Panksepp, J. (1999). High-frequency ultrasonic vocalizations index conditioned pharmacological reward in rats. Physiology & Behavior, 66, 639-643.

Knutson, B. Burgdorf, J., & Panksepp, J. (1997). High frequency ultrasonic vocalizations mark amphetamine preference in rats. Proceedings of the Society for Neuroscience Annual Meeting, New Orleans, 23(2), 1319.

Leshner, A. I. & Koob, G. F. (1999). Drugs of abuse and the brain. Proceedings of the Association of American Physicians, 111, 99-108.

Lorenc-Koci, E., Wolfarth, S., & Ossowska, K. (1996). Haloperidol-increased muscle tone in rats as a model of parkinsonian rigidity. Experimental Brain Research, 109, 268-276.

Lu, W. & Wolf, M. E. (1999). Repeated amphetamine administration alters AMPA receptor subunit expression in rat nucleus accumbens and medial prefrontal cortex. Synapse, 32, 119-131.

McBride, W. J., Murphy, J. M., & Ikemoto, S. (1999). Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behavioral Brain Research, 101, 129-152.

McIntosh, T. K. & Barfield, R. J. (1980). The temporal patterning of 40-60 kHz ultrasonic vocalizations and copulation in the rat (rattus norvegicus). Behavioral and Neural Biology, 29, 349-358.

Paxinos, G. & Watson, C. (1986). The rat brain in stereotaxic coordinates (2nd ed.). New York: Academic Press.

Paxinos, G. & Watson, C. (1997). The rat brain in stereotaxic coordinates (3nd ed.). New York: Academic Press.

Pijnenburg, A. J. J., Honig, W. M. M., Van Der Heyden, J. A. M., & Van Rossum, J. M. (1976). Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. European Journal of Pharmacology, 35, 45-58.

Pinel, J. P. J. (1997). Biopsychology. (Chapter 3). Needham Heights, MA: Allyn & Bacon.

Sales, G. & Pye, D. (1974). Ultrasonic Communication by Animals. London: Chapman and Hall.

Schiorring, E. (1979). An open field study of stereotyped locomotor activity in amphetamine-treated rats. Psycholopharmacology, 66, 281-287.

Stewart, J. & Druhan, J. P. (1993). Development of both conditioning and sensitization of the behavioral activating effects of amphetamine is blocked by the non-competitive NMDA receptor antagonist, MK-801. Psycholopharmacology, 110, 125-132.

Thomas, D. A. & Barfield, R. J. (1985). Ultrasonic vocalization of the female rat (rattus norvegicus) during mating. Animal Behavior, 33, 720-725.

Ungerstedt, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiologica Scandinavica Supplementum, 367, 1-48.

Wolf, M. E. & Khansa, M. R. (1991). Repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. Brain Research, 562, 164-168.

Wu, M., Brudzynski, S. M., & Mogenson, G. J. (1993). Differential effects of quinpirole in the nucleus accumbens depending on the initial level of locomotor activity. Brain Research Bulletin, 32, 395-398.

Table 1

Acoustical Differences Between '50 kHz' and '22 kHz' Calls in Rats

	Call Descriptor	
Acoustic Parameter	50 kHz	22 kHz
Sound Frequency	35 - 70 kHz*	20 - 35 kHz
quality	high	low
Duration	3 - 300 ms	300 - 3000 ms
quality	short	long
Bandwidth	2 - 50 kHz	1 - 5 kHz
quality	wide	narrow
Constant Parameter	relatively constant duration; variable sound frequency	relatively constant sound frequency; variable call duration

Note. Reference: Brudzynski, 1994; Fu & Brudzynski, 1994; Sales, 1974

*In the recent literature high frequency calls are reported to range from 35-70 kHz; however, Sales (1974) reports a summary which indicates calls can be as high as 120 kHz.

Table 2

Description of Testing Conditions and the Number of Rats Used in each Condition

Experimental Conditions	Treatment Description
Experiment 1 (n = 10)	
Sal	ip Sal treatment
Amph	ip AMPH treatment
Experiment 2 (n = 18)	
Group 1 (n = 9)	
Sal/Sal	ic Sal pretreated with ip Sal
Sal/Glu	ic GLU pretreated with ip Sal
LoAmph/Glu	ic GLU pretreated with ip AMPH (1.5mg/kg)
MdAmph/Sal	ic Sal pretreated with ip AMPH (2.0mg/kg)
MdAmph/Glu	ic GLU pretreated with ip AMPH (2.0mg/kg)
HiAmph/Glu	ic GLU pretreted with ip AMPH (2.5mg/kg)
Group 2 (n = 9)	
Sal/Sal	ic Sal pretreated with ip Sal
Sal/Glu	ic GLU pretreated with ip Sal
LoAmph/Sal*	ic Sal pretreated with ip AMPH (1.5mg/kg)
LoAmph/Glu	ic GLU pretreated with ip AMPH (1.5mg/kg)
MdAmph/Sal	ic Sal pretreated with ip AMPH (2.0mg/kg)
MdAmph/Glu	ic GLU pretreated with ip AMPH (2.0mg/kg)
HiAmph/Sal*	ic Sal pretreted with ip AMPH (2.5mg/kg)
HiAmph/Glu	ic GLU pretreated with ip AMPH (2.5mg/kg)
Experiment 3 (n = 9)	
Sal/Glu	ic GLU pretreated with ic Sal
MdAmph/Glu	ic GLU pretreated with ic AMPH (20 ug)
Experiment 4 (n = 10)	
Sal	ic Sal
LoAmph	ic AMPH (10 mg)
HiAmph	ic AMPH (40 ug)
Experiment 5 (n = 20)	
Ctrl/Glu	ic GLU treated with a control
Hal/Glu	ic GLU pretreated with HAL

Note. * for the analyses with these conditions, the data from only 6 rats were available.

Table 3

Range of Acoustical Parameters (Sound Frequency, Call Duration, and Bandwidth) for each Experimental Condition

	Acoustical parameters								
	Peak Frequency (kHz)			Single Call Duration (ms)			Bandwidth (kHz)		N
Experiments	Mean (sd)	min	max	Mean (sd)	min	max	Mean (sd)	min	max
Experiment 1									
Sal	58.28 (3.75)	53.76	63.49	30.53 (8.19)	24.45	45.51	7.43 (1.47)	4.86	8.756
Amph	55.13 (4.25)	49.77	62.11	40.78 (8.55)	29.27	46.60	7.87 (1.01)	6.15	8.908
Experiment 2									
Sal/Sal	53.16 (5.84)	37.28	59.20	34.75 (8.69)	24.22	48.98	6.93 (1.66)	4.40	9.6012
Sal/Glu	54.82 (4.89)	46.27	64.20	37.25 (9.42)	25.78	59.37	7.50 (2.41)	4.50	14.0015
LoAmph/Sal	55.51 (2.94)	49.98	58.67	30.60 (7.31)	21.37	44.84	7.03 (1.81)	4.58	10.307
LoAmph/Glu	56.95 (5.64)	46.03	64.00	36.86 (11.74)	20.24	65.11	6.89 (1.53)	4.07	10.6919
MdAmph/Sal	55.58 (4.35)	46.66	61.95	35.47 (8.27)	19.96	51.75	7.95 (1.73)	3.97	11.7317
MdAmph/Glu	55.95 (3.85)	50.21	65.71	34.83 (9.75)	17.70	56.10	6.94 (1.45)	4.86	9.1220
HiAmph/Sal	54.04 (8.24)	39.66	69.04	33.16 (8.36)	21.58	48.13	6.26 (1.40)	5.04	8.749
HiAmph/Glu	54.48 (4.92)	43.89	63.03	34.77 (7.56)	22.95	50.15	7.79 (1.89)	4.99	12.0218
Experiment 3									
Sal	57.96 (1.85)	55.21	59.95	37.99 (10.32)	23.55	48.14	8.08 (0.94)	6.89	8.877
Sal/Glu	55.00 (3.28)	50.56	60.83	42.95 (17.66)	24.84	73.12	7.97 (1.85)	5.42	12.905
MdAmph	49.93 (4.80)	56.90	60.06	35.74 (5.86)	27.97	38.33	7.07 (0.82)	7.04	11.166
MdAmph/Glu	58.83 (1.96)	43.73	57.09	34.60 (5.76)	25.55	40.63	8.77 (2.13)	5.57	8.003

table 3 continued.

Experiment 4									
Sal	59.47 (3.64)	53.72	63.15	28.95 (3.29)	25.20	33.09	8.09 (1.67)	5.97	10.667
LoAmph	60.21 (3.12)	55.49	63.04	29.11 (1.55)	24.03	33.56	7.23 (1.77)	5.60	9.895
HiAmph	59.11 (3.89)	51.90	63.84	28.80 (4.64)	23.44	35.63	8.10 (1.61)	5.76	10.327
Experiment 5									
Ctrl/Glu	55.18 (5.42)	42.25	61.26	33.97 (11.09)	20.92	64.84	7.21 (1.86)	4.32	11.8712
Hal/Glu	52.14 (5.23)	46.10	55.30	38.66 (6.25)	34.06	45.78	6.91 (0.69)	6.11	7.393

Note. Experiment 1: Systemic AMPH/Sal only; Experiment 2: Intra-AHPOA glutamate pretreated with systemic AMPH/Sal; Experiment 3: Intra-AHPOA glutamate pretreated with Intra-AHPOA AMPH; Experiment 4: Intra-AHPOA glutamate pretreated with higher/lower Intra-AHPOA AMPH; Experiment 5: Intra-AHPOA glutamate (34 mg) pretreated with systemic haloperidol.

Figure 1. Mesolimbic dopamine system. Longitudinal section of the rat brain. Abbreviations are: AH - anterior hypothalamic area, CC - corpus collosum, Ce - cerebellum, CP - caudate-putamen, Hip - hippocampus, Hyp - Hypothalamus, MFB - medial forebrain bundle, MP - medial preoptic area, N.Acc. - nucleus accumbens, Se - septum, SN - substantia nigra, TH - thalamic nucei, OB - olfactory bulb, PfCx - prefrontal cortex., VTA - ventral tegmental area.

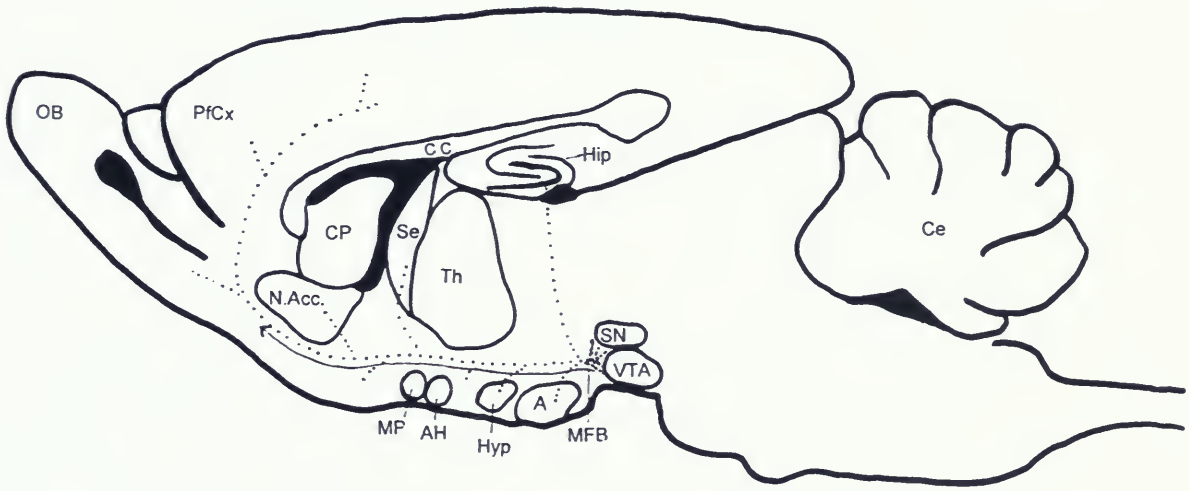

Figure 2 a.& b. Localization of intracerebral injection sites with a) GLU and b) AMPH. Sites from which calls were induced are marked with shaded circles and those from which calls were not induced are marked with open circles. Abbreviations are: AHA - anterior hypothalamic area, BN - bed nucleus of stria terminalis, ca - commissura anterior, CC - corpus collosum, CP - caudate-putamen, DB - diagonal band, ic - capsula interna, LA - lateral hypothalamic area, LP - lateral preoptic area, MP - medial preoptic area, NA - nucleus accumbens, oc - optic chiasm, PA - periventricular hypothalamic nucleus, SE - septum, TH - thalamic nucei, OT - olfactory tubercle, VDB - ventral diagonal band.

Figure 2 a. Intracerebral injections of GLU.

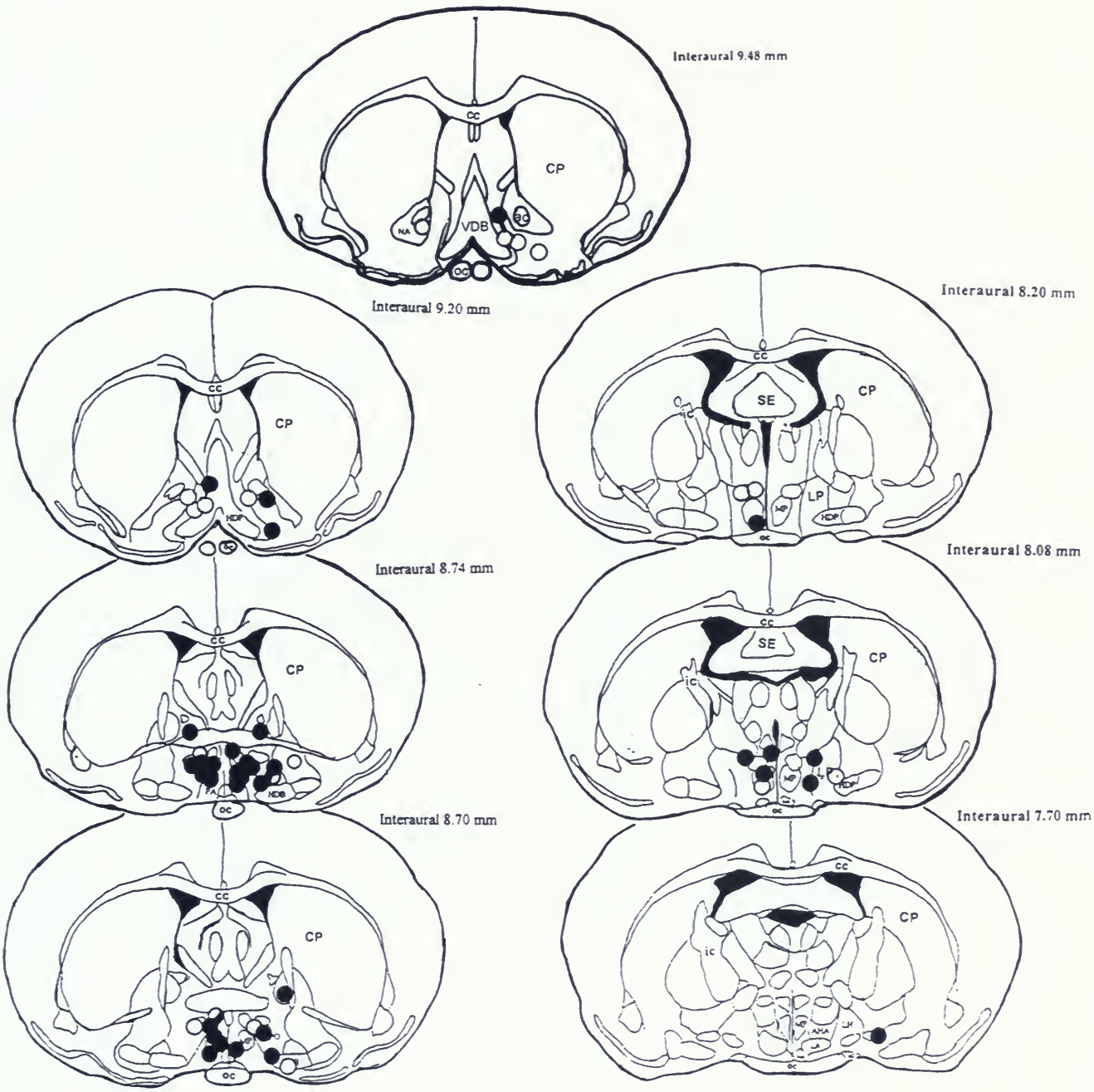

Figure 2 b. Intracerebral injections of AMPH.

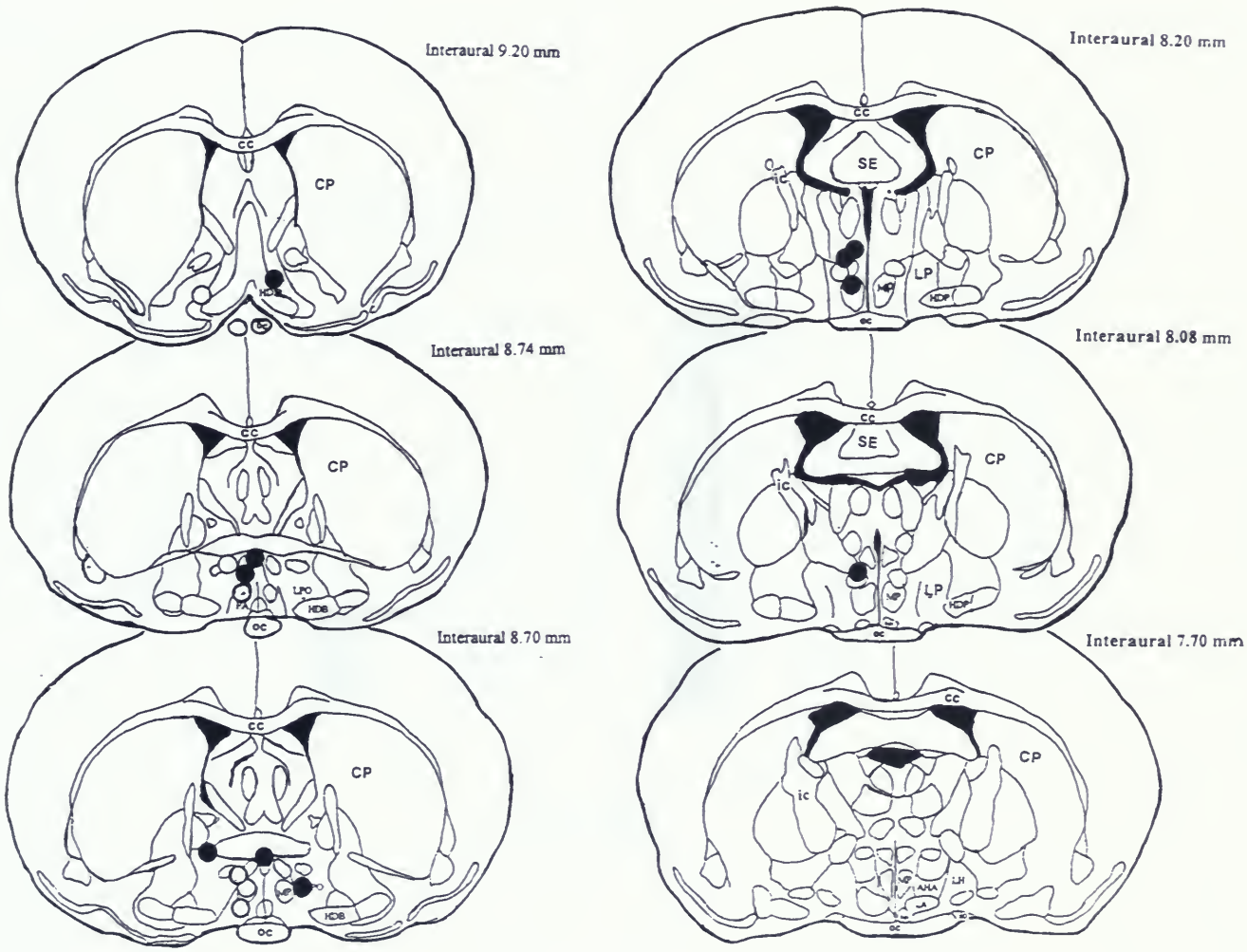

Figure 3. Systemic AMPH and SAL conditions of the mean number of calls, and response latency and duration (Experiment 1). Response latency and duration (min) are along the primary Y axis and the number of calls (transformed) are along the secondary Y axis. Response latency was measured in seconds but converted to minutes for the presentation of this graph. Error bars = SEM. N = 10.

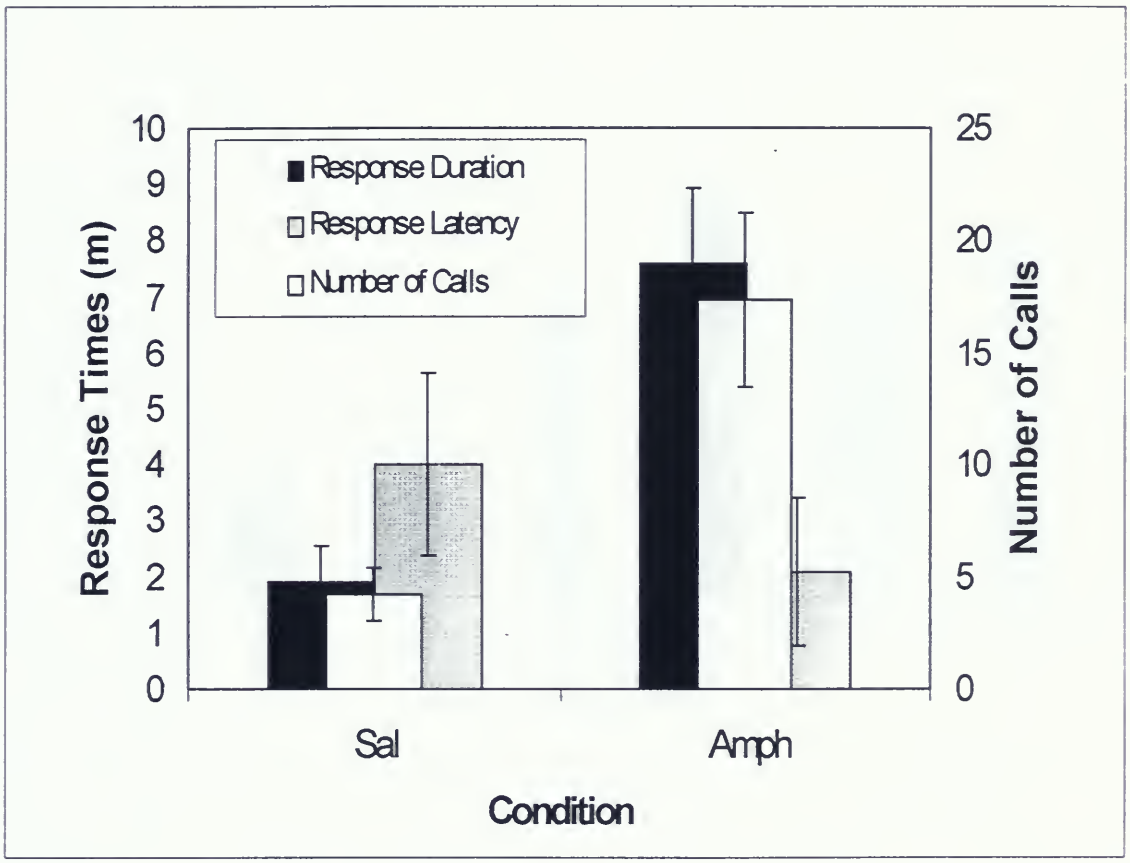

Figure 4 a. Mean number of calls (transformed) emitted with intra-AHPOA GLU (or Sal) pretreated with AMPH (or Sal) (Experiment 2). LoAmph = 1.5 mg/kg of AMPH, MdAmph = 2.0 mg/kg of AMPH, HiAmph = 2.5 mg/kg of AMPH. Error bars = ± SEM. N = 18.

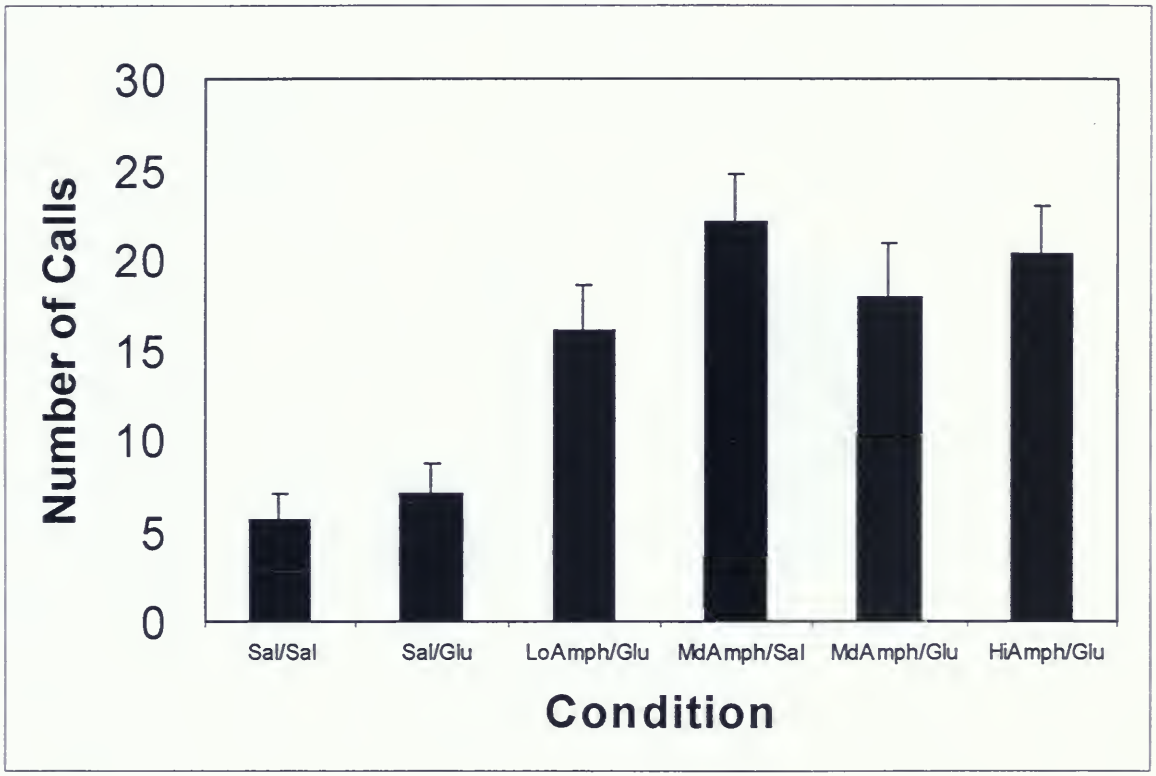

Figure 4 b. Mean number of calls (transformed) emitted after intra-AHPOA GLU (or Sal) pretreated with 2.0 mg/kg of AMPH and Sal (Experiment 2). Error bars = ± SEM. N = 18.

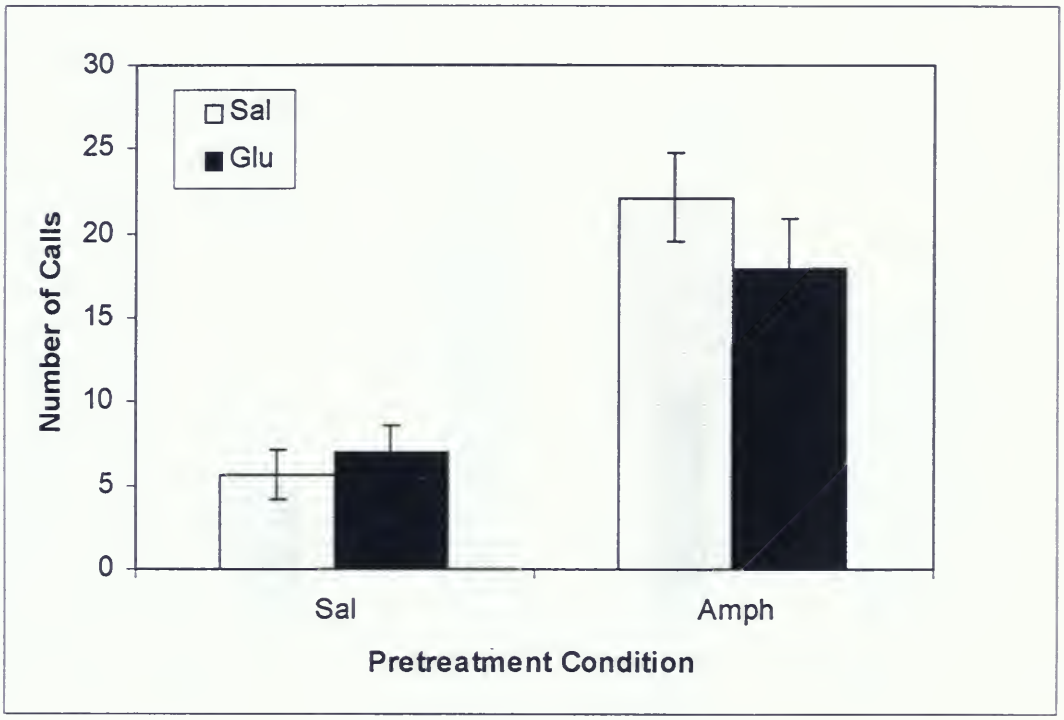

Figure 4 c. Mean number of calls (transformed) emitted after intra-AHPOA GLU (or Sal) pretreated with AMPH (or Sal) for all conditions (Experiment 2). LoAmph = 1.5 mg/kg of AMPH, MdAmph = 2.0 mg/kg of AMPH, HiAmph = 2.5 mg/kg of AMPH. Error bars = ± SEM. N = 6.

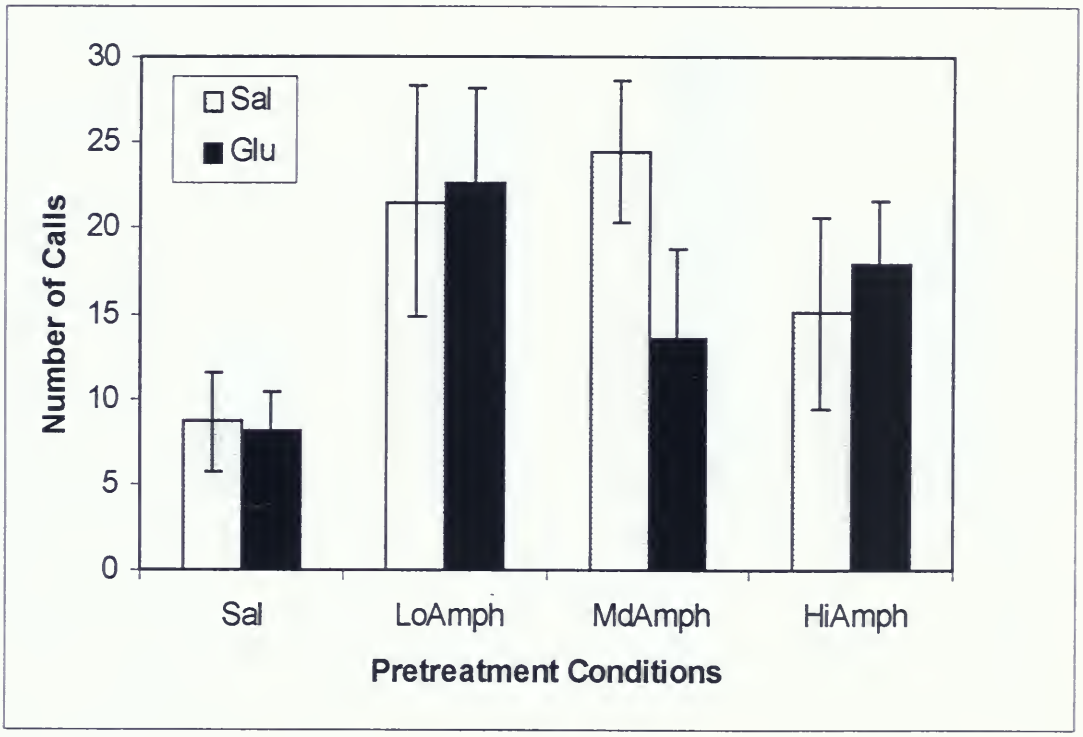

Figure 4 d. Mean number of calls (transformed) for AMPH pretreatment with intra-AHPOA Sal treatment (Experiment 2). LoAmph = 1.5 mg/kg of AMPH, MdAmph = 2.0 mg/kg of AMPH, HiAmph = 2.5 mg/kg of AMPH. Error bars = ± SEM. N = 6.

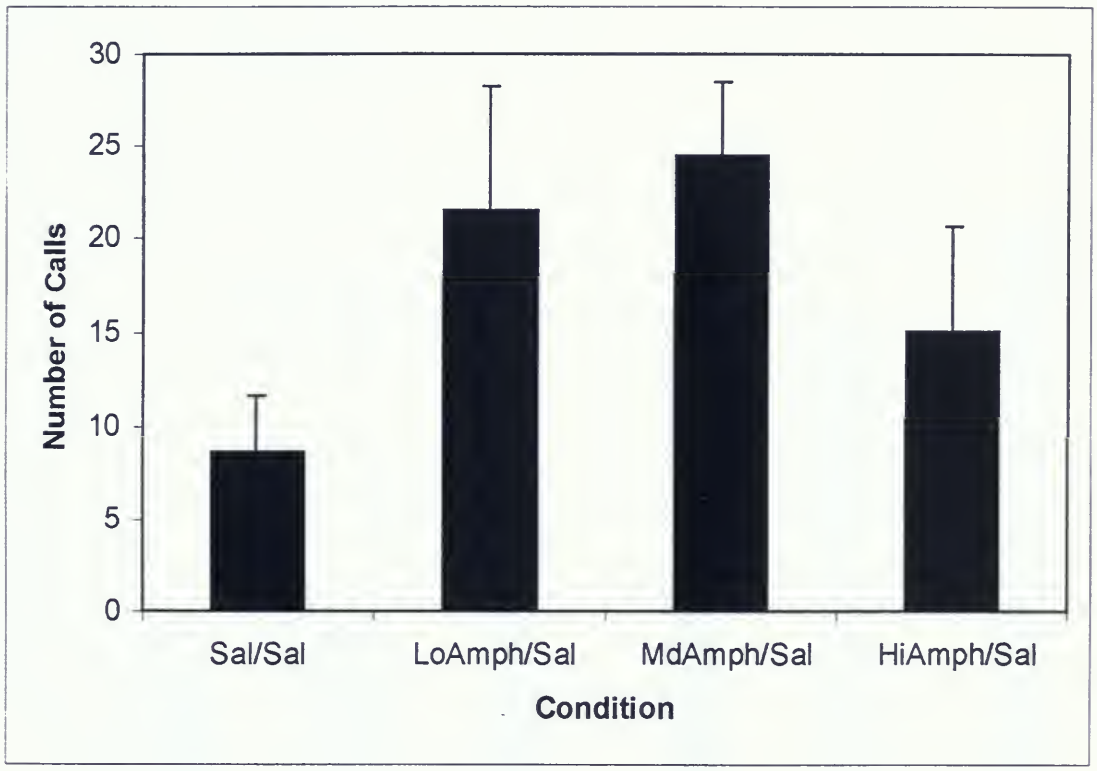

Figure 4 e. Response latency and response duration (min) for intra-AHPOA GLU pretreated (or Sal) with systemic AMPH (or Sal) (Experiment 2). Response latency was measured in seconds but converted to minutes for the purpose of the graph only. LoAmph = 1.5 mg/kg of AMPH, MdAmph = 2.0 mg/kg of AMPH, HiAmph = 2.5 mg/kg of AMPH. Error bars = ± SEM. N = 18.

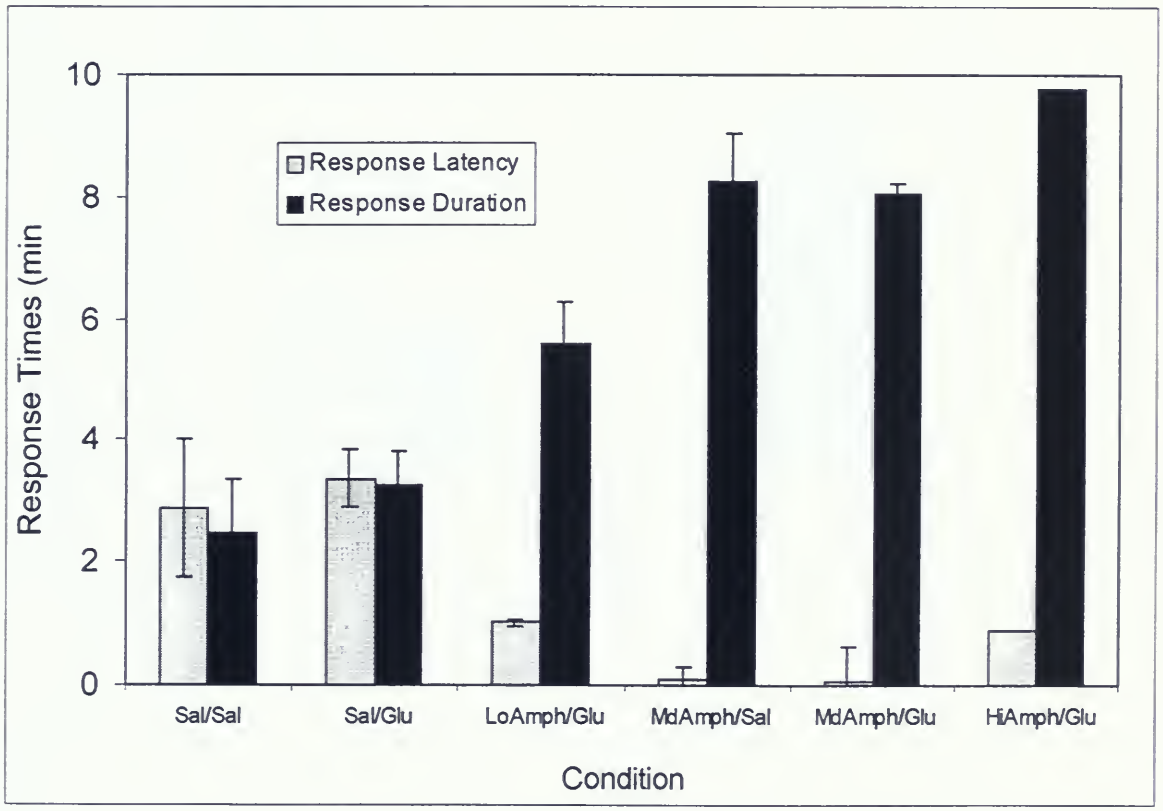

Figure 5 a. Mean number of calls (transformed) for intra-AHPOA glutamate pretreated with intra-AHPOA AMPH or Sal (Experiment 3). Error bars = ± SEM. N = 8.

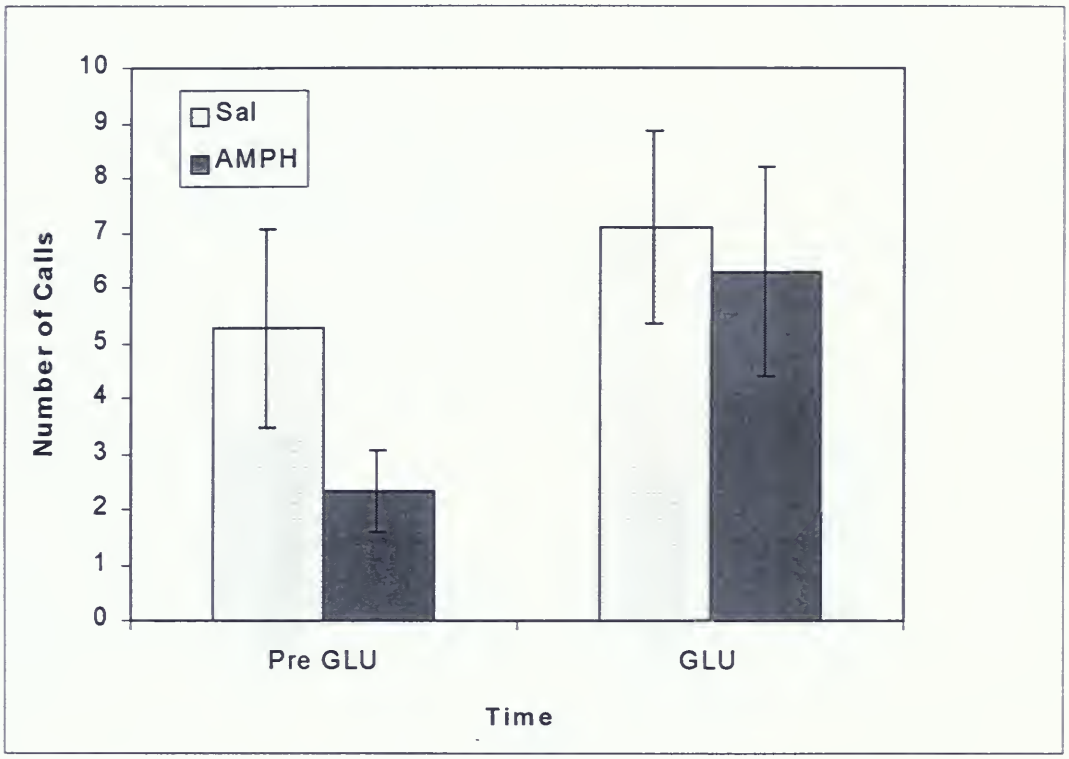

Figure 5 b. Response latency and response duration (min) of intra-AHPOA pretreatments (AMPH and Sal) and treatments (GLU) (Experiment 3). Response latency was converted to minutes from seconds for the purpose of the graph. Error bars = ± SEM. N = 9.

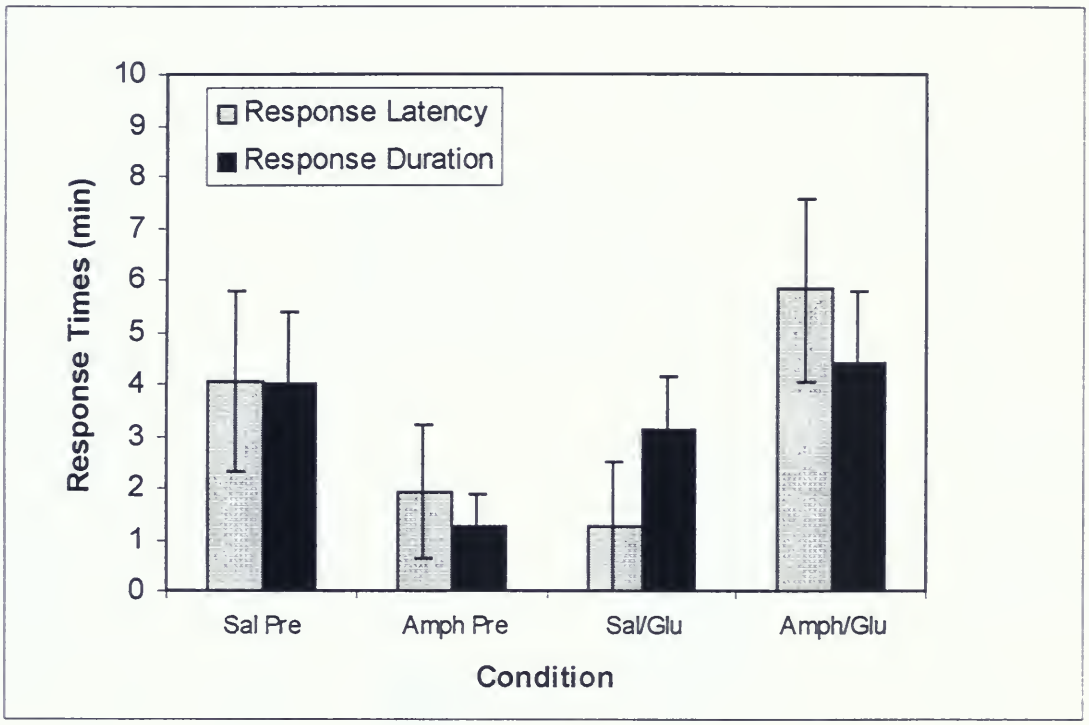

Figure 6. Systemic HAL and SAL conditions of the mean number of calls, and response latency and duration (Experiment 5). Response latency and duration (min) are along the primary Y axis and the number of calls (transformed) are along the secondary Y axis. Response latency was measured in seconds but converted to minutes for the presentation of this graph. Error bars = SEM. N = 20.