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Abstract

Gamma amino-butryic acid (GABA) has been implicated in the stress response. There is conflicting evidence regarding whether GABA inhibits or potentiates stress responding in animals. Much of the research to date suggests GABA has an inhibitory effect on the stress response (Shekhar, Hingtgen, & Dimicco, 1990) However, there is also evidence suggesting that GABA has an excitatory role in stress responding (Hawkins, Baumeister, LaRue, Fountain, Highsmith, Jeffries, & Duke, in review). The purpose of the proposed study was to examine the role of GABA in the stress response. There were two experiments. The first consisted of a group of animals that received four intraventricular injections: saline, and three doses of a GABAa antagonist, bicuculline. A mild tail-pinch was applied to induce stress. During the time the stressor was applied several behavioral observations were recorded. The second experiment evaluated the effect of bicuculline and the GABAa agonist muscimol given simultaneously. Animals received injections of 100ng of muscimol preceded by one of three doses of bicuculline or vehicle control. A time-dose interaction was found for one of the dependent measures in the bicuculline alone study. However, there was no effect of drug on any of the remaining five dependent variables. In addition bicuculline coadministered with muscimol did not have an effect on any of the stress-induced behaviors measured. Attrition is proposed as the most likely cause for the lack of findings in both studies.

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Gamma amino-butyric acid

Gamma amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (Lloyd and Morselli, 1983). GABA works primarily on three receptor complexes; GABAa, GABAb, and GABAc (Wang and Randic, 1994). It is globally distributed throughout the brain and is estimated to be in 60-70% of all synapses in the central nervous system (Fahn, 1976). GABA plays an important role in many different behaviors and physiological functions including locomotor activity, feeding behavior, aggression, sexual behavior, mood, regulation of pain, cardiovascular regulation and thermoregulation (Matsuda, Lolait, Brownstein, Young, and Bonner, 1990). In addition it has been suggested that GABA regulates emotional expression and behavior (Shekhar, Hingtgen, and Dimicco, 1990; Corda, Lecca, Piras, Di Chiara, and Giorgi, 1997).

Animals' responses to stress

A stress response is designed to help an organism adapt to a change and return to a lower state of arousal (Levin and Billington, 1989). Animals' stress responses include several stereotypical behaviors. For example, increases in struggling and defecation are seen in rats exposed to immobilization stress (Ushijima, Mizuki, Hara, Watanabe and Yamada, 1986). Sated rats respond to tail-pinch stress with increased eating, gnawing, licking, locomotion, and vocalization (Antelman, Szechtman, Chin and Fisher, 1975; Hawkins, Fuller, Baumeister, and McCallum, 1994; Hawkins, Baumeister, LaRue, Fountain, Highsmith, Jeffries, and Duke, in review). A similiar phenomenon has been found in mollusks. When the tip of a slug's tail is pinched, it responds by increasing its food intake (Kavaliers, and Hirst, 1985). Rabbits, rats, and monkeys respond to electric shock treatments by increasing their food intake

(Morley, Levine, and Willenbring, 1986). Defeat stress has also been shown to increase food intake in mice (Teskey, Kavaliers, and Hirst, 1984).

Gamma amino-butyric acid and Stereotypy

As mentioned above, stereotypies are commonly evoked by stressors. GABA has been linked to stereotypy. Injections of muscimol (a GABAa agonist) into the raphe nuclei result in an increase in food intake and also elicit gnawing (Klitenick and Wirtshafter, 1989; Przewlocka, Stala, and Scheel-Kruger, 1979). This increase in eating is reversed by injections of bicuculline, a GABAa antagonist (Przewlocka, et al.,1979). Other studies have shown that muscimol injected into the substantia nigra zona reticulata (SN) results in gnawing, escape behavior, sniffing, licking, oral stereotypy, self biting, and vocalization (Baumeister and Frye, 1984; Taha, Dean, and Redgrave, 1982; Scheel-Kruger, 1986; Hawkins, et al., review). Conversely, GABA injected into the mesencephalic reticular formation suppresses oral stereotypy (Dean, Redgrave, and Eastwood, 1982).

Gamma amino-butyric acid and Stress

GABA has also been implicated in the stress response. There is conflicting evidence as to whether GABA inhibits the stress response or potentiates it. The most common view is that GABA's role in the stress response is inhibitory. Several studies support this theory. Shekhar et al.,(1990) suggested that GABA regulates anxiety in rats by acting on GABAa receptors in the posterior hypothalamus. Using a conflict paradigm where animals recieved a shock for positively reinforced behavior (ie. pressing a lever to recieve sweet milk), they found that injections of muscimol into the posterior hypothalamus increased the number of times the lever was pressed. They also found that injections of bicuculline into the same region of the brain decreased the number of times the lever was pressed. Anderson and DiMicco (1990) have suggested that cardiovascular responses to stress may be regulated by GABA in the dorsomedial hypothalamus. They used microdialysis to assess and alter the extracellular levels of GABA in the dorsomedial hypothalamus. Changes in blood pressure and heart rate were

evaluated simultaneously to examine their response to changes in extracellular GABA. They found that increasing GABA in the dorsomedial hypothalamus resulted in a reduction of stress-induced tachycardia. Similarly, Stotz-Potter, Morin, & DiMicco (1996) found that microinjections of muscimol into the dorsomedial hypothalamus of rats attenuated the cardiovascular changes associated with stress. In another study, Keim & Shekhar (1996) examined the effect of bicuculline injected into the dorsomedial hypothalamus on heart rate, blood pressure, and plasma corticosterone levels. Animals were implanted unilaterally with microinjection cannulae and were fitted with femoral arterial catheters to monitor heart rate and blood pressure. They found that injecting bicuculline into the dorsomedial hypothalamus, thereby blocking GABAa receptor mediated inhibition, resulted in an increase of heart rate and blood pressure in addition to an increase of corticosterone secretion. Sun, Li, & Wang (1991) examined the effects of intracerebroventricular injections of GABA on cardiovascular changes. They found that GABA injected into the lateral ventricles decreased heart rate, systolic, diastolic, and mean arterial pressure. In addition, they found that when rats were injected with GABA into the lateral ventricles and were subjected to six hours of continuous immobilization stress, the depressor and bradycardia responses of GABA were attenuated.

In contrast to the studies described above, there is evidence to suggest that GABA potentiates the stress response. Injection of muscimol into the SN increases stereotyped, self-injurious behavior, head nodding, sniffing, locomotion, and gnawing (Baumeister and Frye, 1984; Frye, Baumeister, Croty, Newman, & Kotrla, 1986; Redgrave, Dean, & Taha, 1984). Przewlocka et al., (1979) found that injections of muscimol into the raphe produce an increase of locomotion and stimulate eating in nondeprived rats. Increases in gnawing and nonprandial drinking have also been shown to occur following injections of muscimol into the raphe (Klitenick et al., 1989). Hawkins, et al. (submitted), using a mild tail pinch to induce

stress, found that administration of muscimol into the lateral ventricles, SN, or an area anterior to the SN, increases stress-induced gnawing in rats. These authors also reported that intraventricular injections of muscimol increased other components of the stress response including, vocalization, revolution, oral stereotypy, and eating.

To date no research has examined the effect of bicuculline on responding evoked by tail pinch, nor has research evaluated the possible effect on stress responding of coadministration of muscimol and bicuculline. The present study was designed to examinie these issues.

METHODS

Animals

Male Sprague-Dawley rats (280-320 g) were used (Division of Lab Animal Medicine, L.S.U.). Animals were housed individually in a temperature-controlled room (73-75 °F). Lights were cycled on a 12-hour photoperiod with lights on at 07.00 h. Water and food (PMI Nutrition International) were available ad libitum.

Surgery and Histology

Prior to being placed in a stereotaxic instrument, animals were immobilized by ether inhalation and anesthetized with Ketamine (90 mg/kg, i.m.) and Rompun (5.0 mg/kg, i.m.). Bilateral stainless-steel guide cannulae (22 ga with 30 ga stylets) were placed in the lateral ventricles at 0 mm anterior to bregma, 1.6 mm to either side of the midline and 3.0 mm ventral to the surface of the brain. Stereotaxic cooridinates were taken from the atlas of Pellegrino, Pellegrino, and Cushman (1979). The cannulae were anchored to the skull by four stainless-steel screws and dental acrylic cement. Five days were allowed for the animals to recover.

At the end of testing the animals were sacrificed by ether inhalation and ink (5µl per side) was injected into the ventricles. General histological procedures followed with removal of the brain to check for ink in the fourth ventricle. If no ink was present, sections were taken

through the implant site using a freezing microtome. These were viewed under a microscope to examine the tracks of the cannulae. Data reported are only from animals that had ink in the fourth ventricle or whose frozen sections showed a clear pathway into the ventricle.

Microinjection Procedures

Microinjections began at approximately 11.00 h. On each day of testing an animal was taken from its home cage and restrained in a towel. Stylets were removed, microinjectors were placed in the guide cannulae, and the injection was began. Visual confirmation of the injection was achieved by inspection of the movement of an air bubble through the injection tubing. Afterward the injectors were kept in place for an additional 1 minute to allow for dispersion. Stylets were replaced into the cannulae.

There were two groups of animals tested. One group (n=12) received intraventricular injections of bicuculline methiodide (Sigma Chemical Co.) by way of stainless-steel (30ga) injectors that extended 1 mm past the ventral tip of the guide cannulae. The bicuculline came from lyophilyzed samples reconstituted in sterile normal saline. Doses used were 0.02nmols, 0.05 nmols, 0.2 nmols, and 0.5 nmols as well as a saline control. These doses were derived from pilot research. All doses are expressed as amount administered per side and were delivered in a 1.0 µl volume over a two minute period by means of an infusion pump (Sage). The second group (n=15) of animals received intraventricular injections of bicuculline methiodide using the procedures described above. Immediately afterward the animals in this group received an intraventricular injection of muscimol (100 ng) (Sigma Chemical Co.) delivered in a 1.0 µl volume. The dose of muscimol used was based on pilot research. The paired doses for this second group were as follows:

Bicuculline dose (nmol)	Muscimol dose (ng)
saline	saline
saline	100 ng
0.05 nmol	100 ng
0.2 nmol	100 ng
0.5 nmol	100 ng

Behavioral Measurement

Immediately following microinjection an animal was placed in a wire testing cage with a preweighed (to the nearest 0.01g) amount of rat chow. Water was not made available during testing. Paper towels were placed underneath the cage to collect any spilled food. The tail of the animal was then passed through the bottom of the wire cage and a moderate pinch was applied for 4 minutes using a curved hemostatic forceps padded with rubber tubing. All animals were evaluated for vocalization, oral stereotypy, number of fecal boli, and duration of revolutions. Three four-minute observations occurred: one immediately following injection, one at 30 minutes after injection, and a third at 60 minutes after injection. Two experimenters observed an animal. One recorded the amount of time the animal spent in revolutions by means of a hand held stopwatch and the number of vocalizations by means of a hand held counter. A revolution was defined as a rapid rotation of at least 180°. A vocalization was defined as the start of each high pitched squeal. A second experimenter recorded the amount of time the animal engaged in oral stereotypy and the number of fecal boli. Oral stereotypy was defined as licking, gnawing, chewing, and teeth chattering. In addition to the four behaviors mentioned above, the amount of food eaten or gnawed was measured. The amount eaten was defined as the difference between the original weight of the food and the weight of the food, including spillage collected from beneath the cage. Amount gnawed was defined as the amount of shredded chow collected from the paper towels beneath the cage.

Research Design

A repeated measures design was used in which each animal received vehicle control and all doses of drug. On each experimental day animals were randomly assigned to a drug condition with the following provisions: no more than three animals were assigned to a single dose each day, and every animal received each level of the drug only once during the experiment.

DataAnalysis

The data from behavioral observations were analyzed using a two-factor analysis of variance. The Newman-Keuls test was used to make post hoc comparisons between group means. Alpha was set at 0.05.

Results

Histological Results

Of the twelve animals used in the bicuculline study, only data from seven animals were used. Three animals did not complete the full sequence of testing: two of these were removed as a result of the cannula assemblies becoming dislodged, and a third animal was removed due to tail damage. Data from two additional animals were removed after histological procedures revealed improper implantation of the cannulae.

Of the fifteen animals used in the bicuculline/muscimol study, only the data from six animals were used. Three animals did not complete the full sequence of testing due to dislodgement of the cannula assemblies. The data from three additional animals were removed due to improper cannulae implants.

Effect of bicuculline on stress response behaviors

The effects on stress responses of bicuculline injected into the lateral ventricles are displayed in Figures 1A-6A. Bicuculline did not produce a dose-related or time-related change in

gnawing (Fig. 1A), oral stereotypy (Fig 2A), eating (Fig. 3A), or vocalizations (Fig. 4A). A main effect of time on revolutions was observed [$F(2,12)=7.633;p=.007$] (Fig. 5A). The number of seconds animals spent revolving increased over the three recording intervals. While this effect seems largely attributable to the highest dose of bicuculline, the doses were statistically indistinguishable. In addition, there was a significant interaction between dose of bicuclline and time on the number of fecal boli (see Fig. 6A) [$F=(8,48)=2.328$; $p=.034$]. Post hoc analysis revealed a significant reduction in the number of fecal boli from the 0 minute to the 60 minute reading. No significant post hoc differences among the doses were detected.

Effect of muscimol coupled with bicuculline on stress response behaviors

Stress responding following coadministration of bicuculline and muscimol is depicted in Figures 1B-6B. As was seen following injections of bicuculline alone, coadministration of bicuclline and muscimol did not alter gnawing (Fig. 1B), oral stereotypy (Fig. 2B), eating (Fig. 3B), or vocalizations (Fig. 4B). Nor did it affect seconds of revolution (Fig. 5B), or number of fecal boli (Fig. 6B).

Discussion

There is evidence that GABA plays a role in the stress response (Shekhar et. al, 1990). However, there is conflicting evidence regarding the nature of this role. Some studies have shown GABA to inhibit stress responding in animals. For example Stotz-Potter et al., (1996) found that intrahypothalamic muscimol reduced heart rate changes associated with stress. Similarly, Keim, & Shekhar (1996) reported that intrahypothalamic bicuculline produced an increase in heart rate and corticosterone levels. In contrast there have been studies which have shown GABA to play an excitatory role in the stress response. Central injections of muscimol have been shown to increase stereotypies and stress-invoked responding (Baumeister and Frye, 1984; Hawkins et al., in review). Prior to the present study no research has examined the effect of bicuculline on stress responding induced by tail-pinch nor has there been any research to

examine the effect of coadministration of bicuculline and muscimol on stress responding induced by tail-pinch.

In the first of the studies reported here, the effect of bicuculline on stress responding was evaluated. A time x dose interaction for the number of fecal boli was found. A time-related increase in the amount of time the rats spent in revolutions was also found. However, the dose of drug was not a factor and therefore this finding is irrelevant to the central issue of GABA-mediation of stress. Bicuculline did not alter the amount of gnawing, eating, oral stereotypies, or vocalizations produced as compared to rats treated with saline. Given these results, this study provides no compelling evidence that GABA has a direct effect on stress responding. The lack of an effect in the bicuculline study may have been due to attrition which led to a decrease in the number of animals per cell. In the future, to account for the possibility of attrition, it would be advisable to start with a larger n. Another possibility is that the doses chosen were not high enough to elicit a response. However, based upon pilot research conducted in this lab, doses at higher levels than those used in this study produce seizures. Perhaps the use of a GABAa antagonist with less seizure-evoking potential (such as the non-competitive antagonist picrotoxin.) may be appropriate

In the second study, reported here the effect of ICV injections of bicuculline coadministered with muscimol on the stress response was evaluated. Stress responding was not altered by injections of bicuculline coadministered with muscimol. There was no effect on any of the dependent variables tested. In addition, muscimol when coadministered with saline, did not produce an increase in stress responding. This last finding is inconsistent with previous studies that have shown muscimol to potentiate the stress response when injected into the lateral ventricles (Hawkins, et al., in review). The results from this finding also do not conform with other reports of muscimol inhibition of stress responding (Stotz-Potter, et al., 1996). The lack of an effect may have also been due to attrition. As with the bicuculline study, the bicuculline

doses used may not have been effective. Additionally there is evidence for a possible ceiling effect occurring. The rats receiving saline only in the present study engaged in as much stereotypy as rats receiving muscimol in a previous study (Hawkins et al., in review). For example, rats receiving saline alone in the present study gnawed an average of 6.42 +/- 3.40 grams of food compared to rats injected with 100ng of muscimol in a previous study that gnawed an average of 10.80 +/- 3.40. grams of food. In addition saline animals in the present study ate 0.45 +/-0.31 grams of food whereas rats receiving muscimol ate 0.73+/-0.22. grams of food. This heightened level of responding could have masked an increase in responding but, of course, would not have affected evaluation of a decrease.

Figure 1

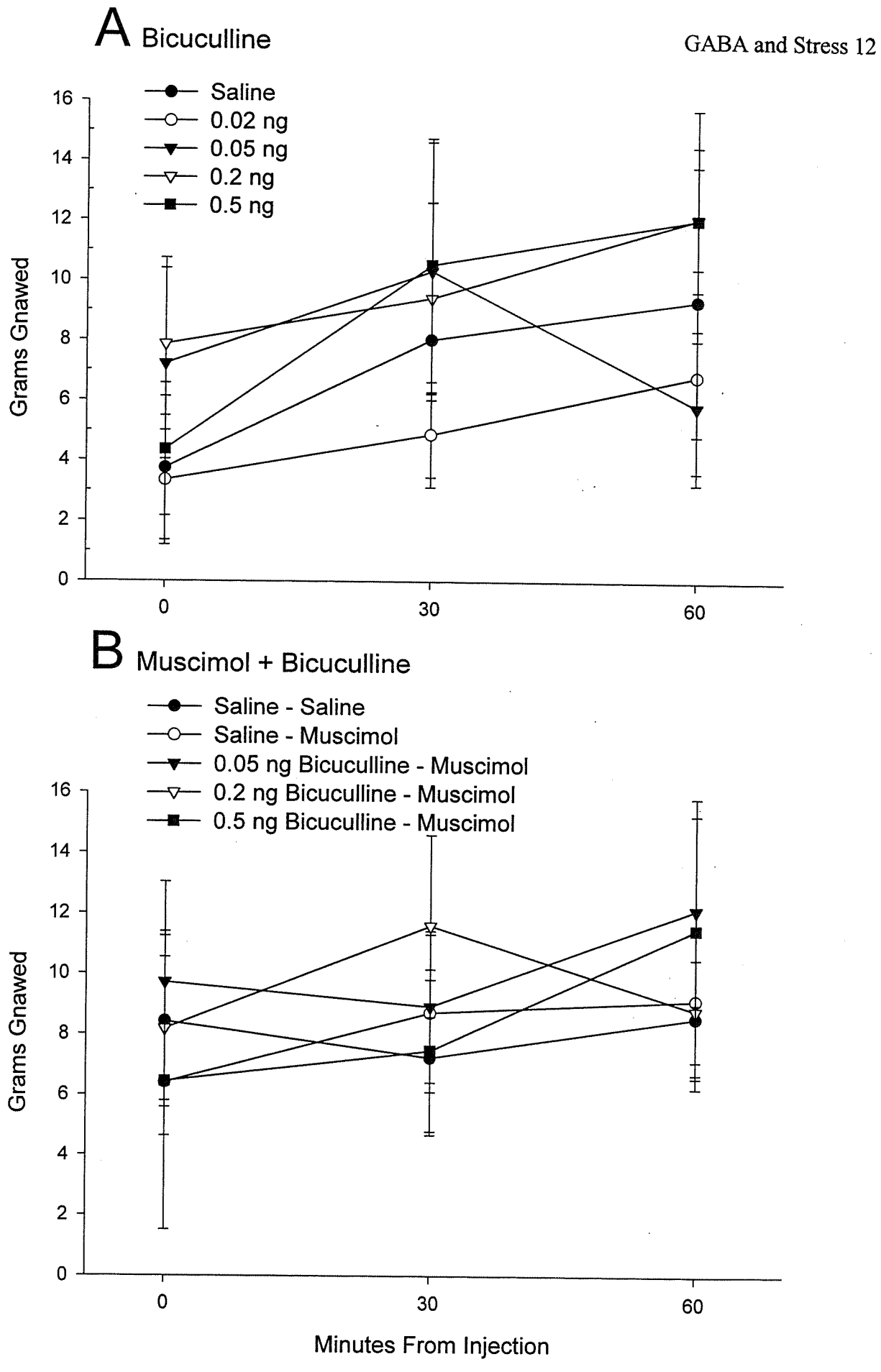

A Bicuculline
Saline
0.02 ng
0.05 ng
0.2 ng
0.5 ng
Grams Gnawed
16
14
12
10
8
6
4
2
0
0
30
60
B Muscimol + Bicuculline
Saline - Saline
Saline - Muscimol
0.05 ng Bicuculline - Muscimol
0.2 ng Bicuculline - Muscimol
0.5 ng Bicuculline - Muscimol
Grams Gnawed
16
14
12
10
8
6
4
2
0
0
30
60
Minutes From Injection

Figure 2

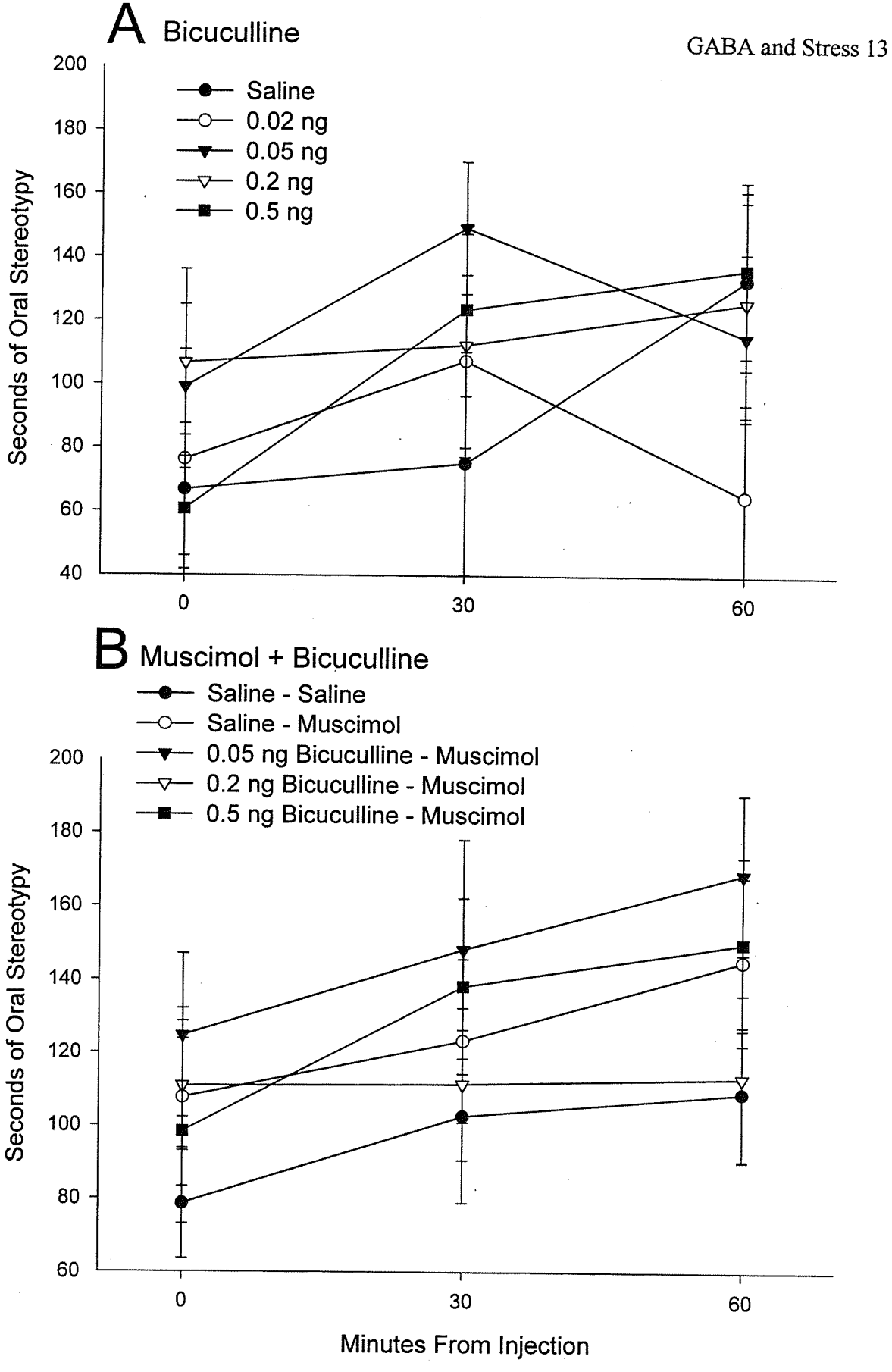

A Bicuculline
Saline
0.02 ng
0.05 ng
0.2 ng
0.5 ng
Seconds of Oral Stereotypy
200
180
160
140
120
100
80
60
40
0
30
60
B Muscimol + Bicuculline
Saline - Saline
Saline - Muscimol
0.05 ng Bicuculline - Muscimol
0.2 ng Bicuculline - Muscimol
0.5 ng Bicuculline - Muscimol
Seconds of Oral Stereotypy
200
180
160
140
120
100
80
60
0
30
60
Minutes From Injection

Figure 3

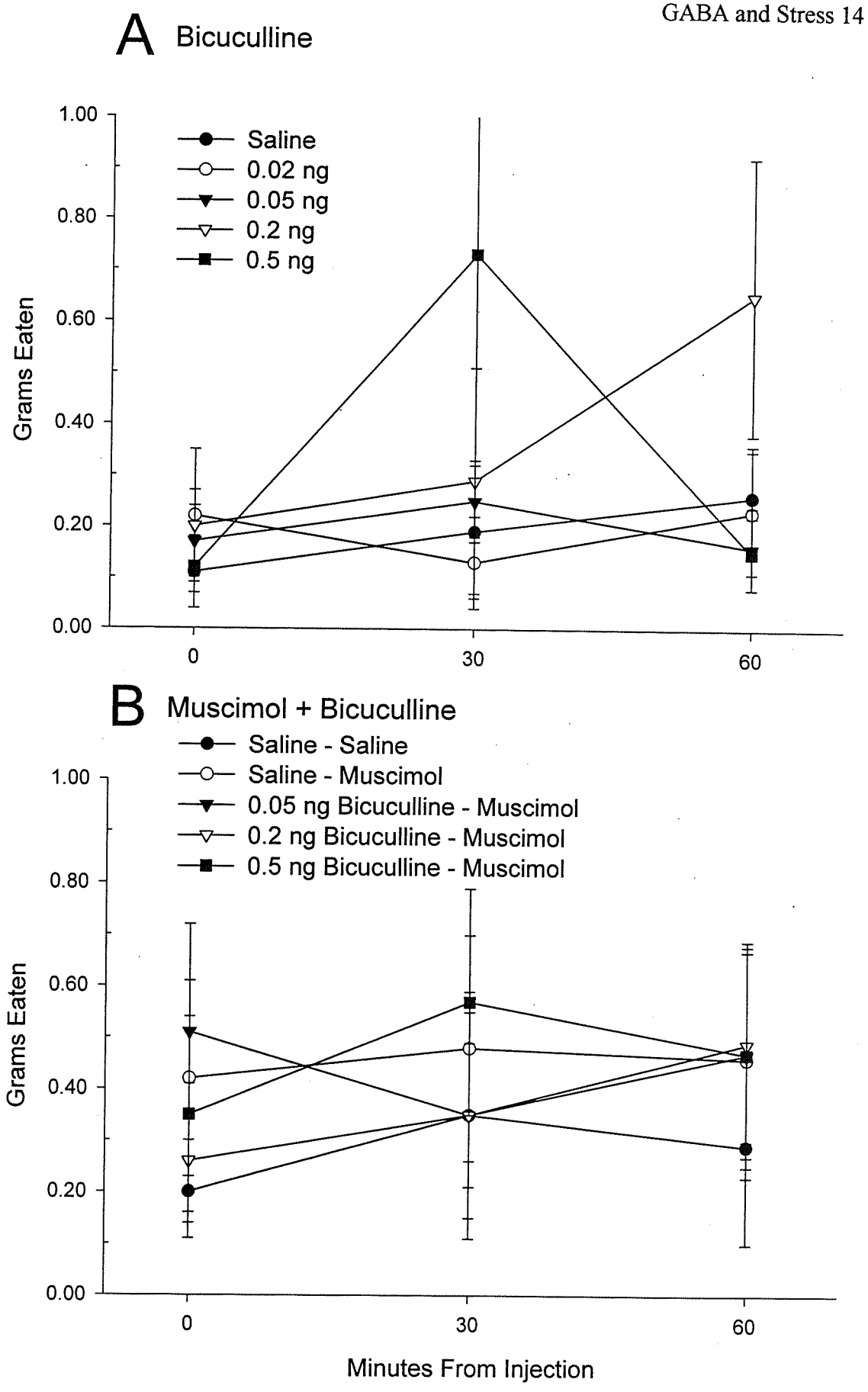

A Bicuculline
Saline
0.02 ng
0.05 ng
0.2 ng
0.5 ng
Grams Eaten
1.00
0.80
0.60
0.40
0.20
0.00
0
30
60
B Muscimol + Bicuculline
Saline - Saline
Saline - Muscimol
0.05 ng Bicuculline - Muscimol
0.2 ng Bicuculline - Muscimol
0.5 ng Bicuculline - Muscimol
Grams Eaten
1.00
0.80
0.60
0.40
0.20
0.00
0
30
60
Minutes From Injection

Figure 4

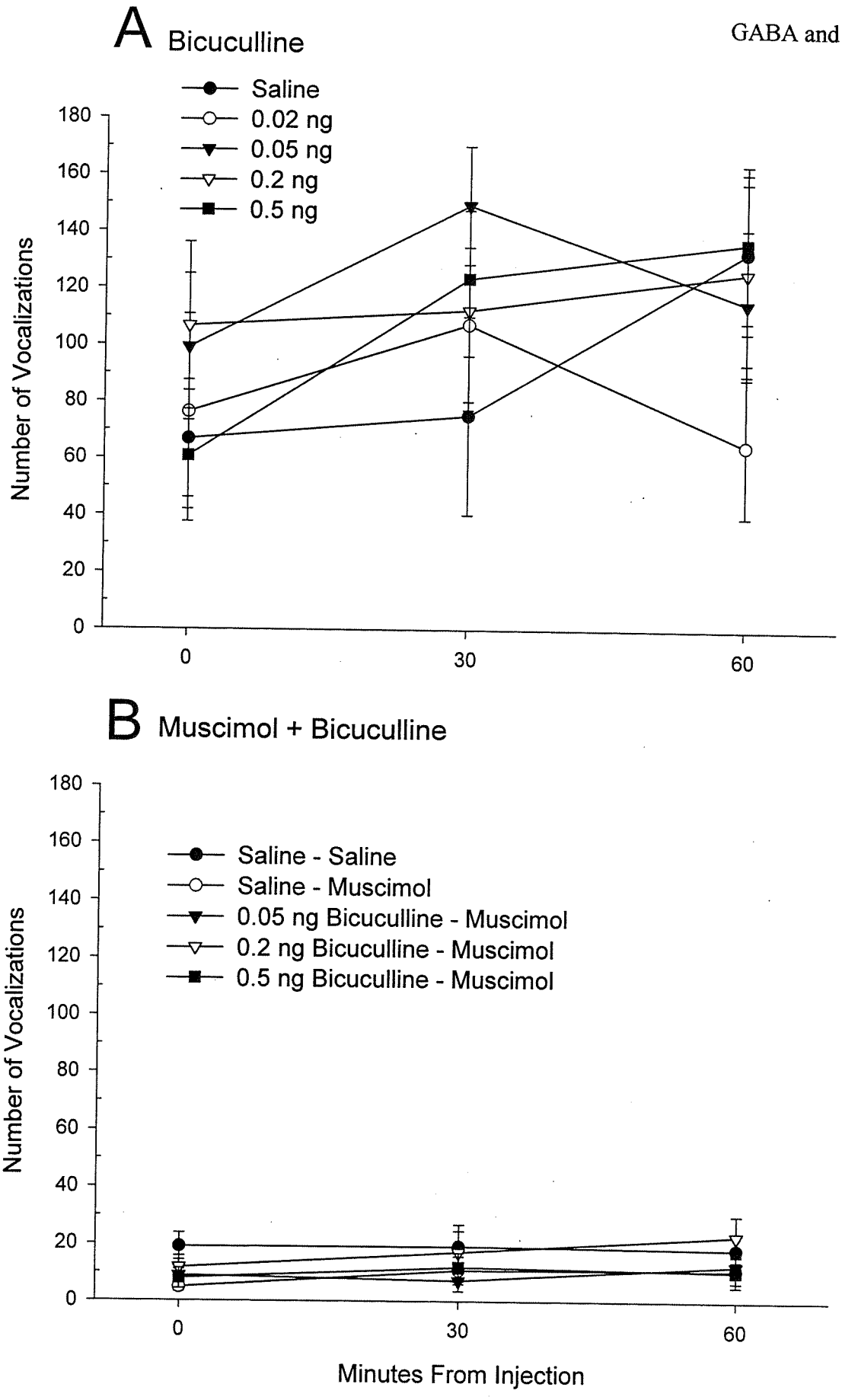

A Bicuculline
Saline
0.02 ng
0.05 ng
0.2 ng
0.5 ng
Number of Vocalizations
180
160
140
120
100
80
60
40
20
0
0
30
60
B Muscimol + Bicuculline
Saline - Saline
Saline - Muscimol
0.05 ng Bicuculline - Muscimol
0.2 ng Bicuculline - Muscimol
0.5 ng Bicuculline - Muscimol
Number of Vocalizations
180
160
140
120
100
80
60
40
20
0
0
30
60
Minutes From Injection

Figure 5

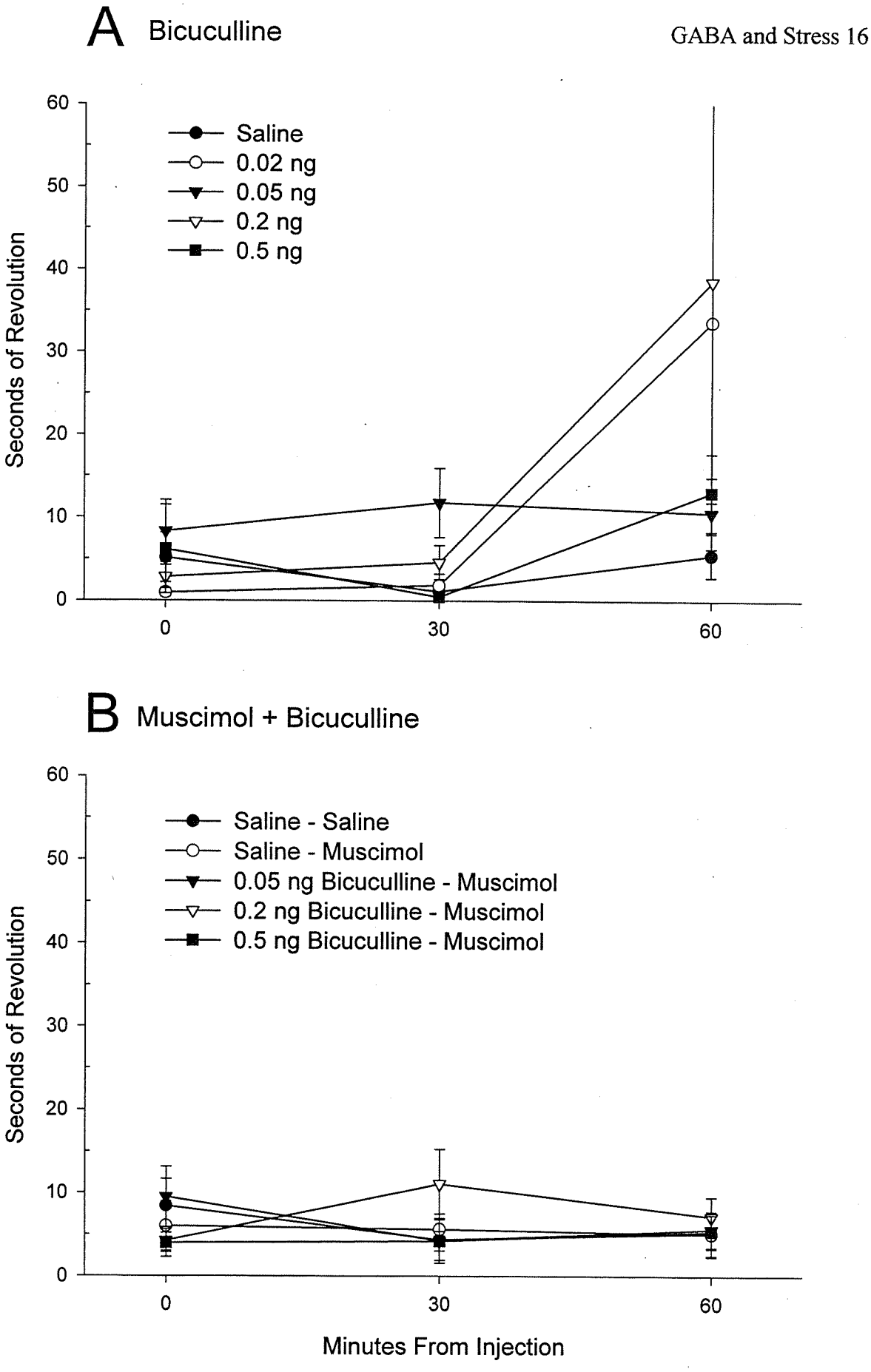
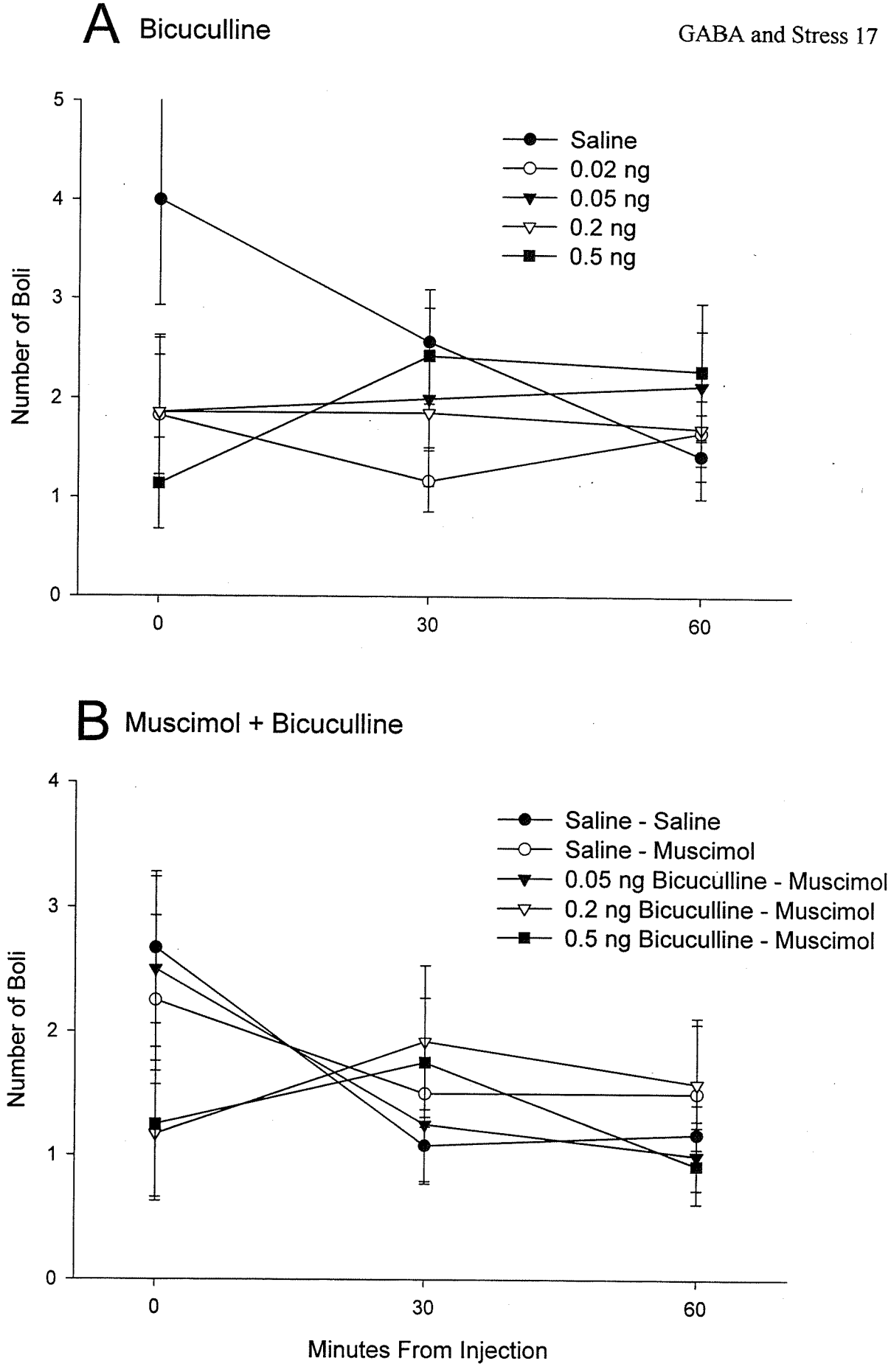

A Bicuculline
Saline
0.02 ng
0.05 ng
0.2 ng
0.5 ng
Seconds of Revolution
60
50
40
30
20
10
0
0
30
60
B Muscimol + Bicuculline
Saline - Saline
Saline - Muscimol
0.05 ng Bicuculline - Muscimol
0.2 ng Bicuculline - Muscimol
0.5 ng Bicuculline - Muscimol
Seconds of Revolution
60
50
40
30
20
10
0
0
30
60
Minutes From Injection

Figure 6


A Bicuculline
Saline
0.02 ng
0.05 ng
0.2 ng
0.5 ng
Number of Boli
0
1
2
3
4
5
0
30
60
B Muscimol + Bicuculline
Saline - Saline
Saline - Muscimol
0.05 ng Bicuculline - Muscimol
0.2 ng Bicuculline - Muscimol
0.5 ng Bicuculline - Muscimol
Number of Boli
0
1
2
3
4
0
30
60
Minutes From Injection

References

Anderson, J.J., & DiMicco, J.A. (1990). Effect of local inhibition of γ-aminobutyric acid uptake in the dorsomedial hypothalamus on extracellular levels of γ-aminobutyric acid and stress-induced tachycardia: A study using microdialysis. The Journal of Pharmacology and Experimental Theraputics, 255(3), 1399-1407.

Antelman, S.M., Szechtman, H., Chin, P., & Fisher, A.E. (1975). Tail-pinch induced eating, gnawing, and licking behavior in rats: Dependence on the nigrostriatal dopamine system. Brain Research, 99, 319-337.

Corda, M.G., Lecca, D., Piras, G., DiChiara, G., & Giorgi, O. (1997). Biochemical parameters of dopaminergic and GABAergic neurotransmission in the CNS of Roman high-avoidance rats and Roman low-avoidance rats. Behavior Genetics, 27, 527-536.

Dean, P., Redgrave, P., & Eastwood, L. (1982). Suppression of apomorphine-induced oral stereotypy in rats by microinjection of muscimol into midbrain. Life Sciences, 30, 2171-2179.

Fahn, S. (1976). GABA. In T. Chase & D. Tower (Ed.), GABA in nervous system function (pp. 169-174). New York: Raven Press.

Frye, G.D., Baumeister, A.A., Crotty, K., Newman, K.D., & Kotrola, K.D. (1986). Evaluation of the role of antinociception in self-injurious behavior following intranigral injection of muscimol. Neuropharmacology, 25, 717-726.

Frye, G.D., Baumeister, A.A., Crotty, K., Newman, K.D., & Kotrola, K.D. (1986). Evaluation of the role of antinociception in self-injurious behavior following intranigral injection of muscimol. Neuropharmacology, 25, 717-726.

Hawkins, M.F., Baumeister, A.A., Larue, R.H., Fountain, L.T., Highsmith, R.W., Jeffries, S.K., & Duke, M.A. (1998). Central GABA activation and behaviors evoked by stress in the rat. Manuscript submitted for publication.

Hawkins, M.F., Fuller, R.D., Baumeister, A.A., & McCallum, M.D. (1994). Effects in the rat of intranigral morphine and DAGO on eating and gnawing induced by stress. Pharmacology, Biochemistry and Behavior, 49, 737-740.

Kavaliers, M., & Hirst, M. (1986). Naloxone-reversible stress-induced feeding and analgesia in the slug limax maximus. Life Science, 38, 203-209.

Keim, S.R., & Shekhar, A. (1996). The effects of GABAa receptor blockade in the dorsomedial hypothalamic nucleus on corticotrophin (ACTH) and corticosterone secretion in male rats. Brain Research, 739, 46-51.

Klitenick, M.A., & Wirtshafter, D. (1989). Elicitation of feeding, drinking, and gnawing following microinjections of muscimol into the median raphe nucleus of rats. Behavioral and Neural Biology, 51, 436-441.

Levine, A.S., Billington, C.J. Stress and consumatory behavior. In Y. Tache, J.E. Morley, and M.R. Brown (Ed.), Neuropeptides and Stress (pp. 188-198). New York: Springer-Verlag.

Lloyd, K.G., & Morselli, F.L. (1983). Psychopharmacology of GABAergic drugs. In H.Y. Meltzer (Ed.), Psychopharmacology, the third generation of progress (pp. 183-203). New York: Raven Press.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., & Bonner, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature, 346, 561-564.

Morley, J.E., Levine, A.S., & Willenbring, M.L. (1986). Stress-induced feeding disorders. In M.O. Carruba & J.E. Blundell (Ed.), Pharmacology of Eating Disorders: Theoretical and clinical developments (pp. 71-99). New York: Raven Press.

Pellengrino, L.J., Pellengrino, A.S., & Cushman, A.J. (1979). A Stereotaxic Atlas of the Rat Brain. New York: Plenum Press.

Przewlocka, B., Stala, L., & Scheel-Kruger, J. (1979). Evidence that GABA in lthe nucleus dorsalis raphe induces stimulation of locomotor activity and eating behavior. Life Sciences, 25, 937-946.

Redgrave, P., Dean, P., & Taha, E.B. (1984). Feeding induced by injections of muscimol into the substantia nigra of rats: unaffected by haloperidol but abolished by large lesions of the superior colliculus. Neuroscience, 13, 77-85.

Scheel-Kruger, J. (1986). Dopamine-GABA interactions: Evidence that GABA transmits, modulates, and mediates dopaminergic functions in the basal ganglia and the limbic system. Acta Neurologics Scandinavia Supplementum, 73, 1-54.

Shekhar, A., Hingten, J.N., & DiMicco, J.A. (1990). GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats. Brain Research, 512, 81-88.

Stotz-Potter, E.H., Morin, S.M., DiMicco, J.A. (1996). Effect of microinjection of muscimol into the dorsomedial or paraventricular hypothalamic nucleus on air stress-induced neuroendocrine and cardiovascular changes in rats. Brain Research, 742, 219-224.

Sun, An-Yang., Li, De-Xing., Wang, Yu-Ling. (1991). Stress-induced changes of central GABAergic function in rats. Acta Pharmacologica Sinica, 12, 387-390.

Taha, E.B., Dean, P., & Redgrave, P. (1982). Oral behavior induced by intranigral muscimol is unaffected by haloperidol but abolished by large lesions of the superior colliculus. Psychopharmacology, 77, 272-278.

Ushijima, I., Mizuki, Y., Hara, T., Kudo, R., Watanabe, K., & Yamada, M. (1986). The role of adenosinergic, GABAergic and benzodiazepine systems in hyperemotionality and ulcer formation in stressed rats. Pyschopharmacology, 89, 472-476.

Wang, R.A., Randic, M. (1994). Activation of mu-opiod receptor modulates GABAa receptor-mediated currents in isolated spinal horn neurons. Neuroscience Letters, 180, 109-113.

Figure Caption

Figure 1. Mean number of grams gnawed over time following central injections of bicuculline (panel A) and central injections of bicuculline paired with muscimol (panel B).

Figure 2 Mean number of seconds spent in oral stereotypy over time following central injections of bicuculline (panel A) and central injections of bicuculline paired with muscimol (panel B).

Figure 3 Mean number of grams eaten over time following central injections of bicuculline (panel A) and central injections of bicuculline paired with muscimol (panel B).

Figure 4 Mean number of vocalizations over time following central injections of bicuculline (panel A) and central injections of bicuculline paired with muscimol (panel B).

Figure 5 Mean number of seconds animals revolved over time following central injections of bicuculline (panel A) and central injections of bicuculline paired with muscimol (panel B).

Figure 6 Mean number of fecal boli over time following central injections of bicuculline (panel A) and central injections of bicuculline paired with muscimol (panel B).