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Effects of co-administration of 2-arachidonylglycerol (2-AG) and a selective μ-opioid receptor agonist into the nucleus accumbens on high-fat feeding behaviors in the rat

Kyle E. Parker[1,2,3], **Jordan G. McCall**[1,2], **Sophia R. McGuirk**[1,2], **Seema Trivedi**[1,2], **Dennis K. Miller**[1,2], and **Matthew J. Will**[1,2]

[1]Department of Psychological Sciences, University of Missouri, Columbia, MO

[2]Christopher Bond Life Sciences Center, University of Missouri, Columbia, MO

[3]Interdisciplinary Neuroscience Program, University of Missouri, Columbia, MO

Abstract

Previous research has demonstrated that the nucleus accumbens is a site where opioids and cannabinoids interact to alter feeding behavior. However, the influence of the endocannabinoid 2-arachidonylglycerol (2-AG) on the well-characterized model of intra-accumbens opioid driven high-fat feeding behavior has not been explored. The present experiments examined high-fat feeding associated behaviors produced by the interaction of 2-AG and the μ-opioid receptor agonist DAla2,N,Me-Phe4,Gly-ol5-enkaphalin (DAMGO) administered into the nucleus accumbens. Sprague-Dawley rats were implanted with bilateral cannulae aimed at the nucleus accumbens and were co-administered both a sub-threshold dose of 2-AG (0 or 0.25μg/0.5μl/side) and DAMGO (0, 0.025μg or 0.25μg/0.5μl/side) in all dose combinations, and in a counterbalanced order. Animals were then immediately allowed a 2hr-unrestricted access period to a palatable high-fat diet. Consumption, number and duration of food hopper entries, and locomotor activity were all monitored. DAMGO treatment led to an increase in multiple behaviors, including consumption, duration of food hopper entry, and locomotor activity. However, combined intra-accumbens administration of DAMGO and a subthreshold dose of 2- AG led to a significant increase in number of food hopper entries and locomotor activity, compared to DAMGO by itself. The results confirm that intra-accumbens administration of subthreshold dose of the endogenous cannabinoid 2-AG increases the DAMGO-induced approach and locomotor behaviors associated with high-fat feeding.

Corresponding author address: Matthew J. Will, Ph.D., Department of Psychological Sciences, University of Missouri, Columbia, Christopher Bond Life Sciences Center, 1201 Rollins St. Columbia, MO 65211, willm@missouri.edu.

We. Examined Effect Of Opioid And The Endocannabinoids On high---fat feeding behaviours.
DAMGO And 2---Ag Were co---administered into the nucleus accumbens prior to feeding. Together They Increased Approach And Locomotor Activity Compared To Each Drug Alone.

Keywords

nucleus accumbens; 2-arachidonylglycerol; palatable food; cannabinoid; DAMGO; opioid; approach; consumption; reward; feeding; high fat

1. Introduction

The latest data indicate that 34.9% of the adult and 16.9% of the child U.S. population is obese (Ogden et al., 2014). Although obesity is certainly the result of a complex set of factors, including sedentary lifestyle, economic factors, genetic predisposition, and stressful life events, the over consumption of calorically dense palatable foods is involved. Research examining the nature of this critical behavior of overconsumption has revealed a distributed feeding network that includes many key regions of the brain and many neuromodulators that may contribute (Will et al., 2003; Berthoud 2012, for review). The nucleus accumbens in particular, and its associated circuitry, is a critical region that mediates the response to palatable food, most notably the actions of opioids (Pecina & Berridge, 2000; Will et al., 2003). For example, administration of the selective μ-opioid agonist D-Ala2, NMe-Phe4, Glyol5-enkephalin (DAMGO) into the nucleus accumbens produces a robust binge-like consumption of palatable diets such as those high in fat and/or sugar (Zhang et al., 2000; Pecina & Berridge, 2000). Behavioral and pharmacological characterizations of intra-accumbens DAMGO suggest that it does not induce a state of negative energy balance (i.e. “hunger”) (Hanlon et al., 2004; Will et al., 2009). Indeed, evidence suggests that the activation of the accumbens with DAMGO acts to increase the hedonic or rewarding nature of the food independent of negative energy balance, in turn producing increased consumption and associated food seeking behaviors (Kelley et al., 2002; Pecina & Berridge, 2000; Will et al., 2009).

Overlapping with the comparably longer period of research on opioids (Bodnar, 2013), research on endocannabinoids has led to promising targets that could lead to therapeutic advancements in the treatment of both obesity and drug addiction (Isoldi and Aronne, 2008; Bermudez-Silva et al., 2010). Systemic activation of the endocannabinoid system produces many of the same behavioral effects as the opioid system, including increased feeding behavior, and reinforcement of drug self-administration behavior (Maldonado and Rodriguez de Fonseca, 2002; Tanda and Goldberg, 2003; Silvestri and Di Marzo, 2013; Cristino et al., 2014; Jager and Witkamp, 2014). The endocannabinoids 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995) and anandamide (Devane et al., 1992) have both been shown to increase feeding behavior when administered into the nucleus accumbens (Kirkham et al., 2002; Mahler et al., 2007). However, the combined influence of these endocannabinoids and opioid receptor agonists has not been explored in a palatable feeding model. It has been demonstrated that the behavioral effects of cannabinoids are partially dependent on co-activation of the opioid system (Williams and Kirkham, 2002; Maldonado and Rodriguez de Fonseca, 2002; Justinova et al., 2004; Skelly et al., 2010). For example, feeding increased by striatal infusions of cannabinoid and opioid agonists is blocked by prior administration of opioid and cannabinoid receptor antagonists, respectively (Williams and Kirkham, 2002; Skelly et al., 2010). Also, sub-threshold doses of

opioid and cannabinoid antagonists that have little or no effect on feeding independently, demonstrate a potentiated effect when administered together (Kirkham and Williams, 2001; Chen et al., 2004; Tallett et al., 2009). Finally, intra-accumbens administration of a subthreshold dose of a selective CB1 agonist WIN55212-2 and DAMGO increased high-fat feeding above levels produced by DAMGO alone (Skelly et al., 2010).

The present study was designed to examine the potential interaction of the opioid and cannabinoid systems within the well-characterized model of intra-accumbens opioid-induced high-fat feeding. Specifically, a sub-threshold dose of 2-AG and multiple near-threshold doses of the μ-opioid agonist DAMGO were co-administered into the nucleus accumbens and multiple behaviors associated with high fat feeding were assessed, including food-directed approach (food hopper entries), consumption, and locomotor activity. Assessing approach, as well as consumption behaviors, has been shown to be critical in understanding the diverse effect of the interaction of opioids and cannabinoids (Tallett et al., 2009). Also, increased food-directed approach responses do not always predict a parallel increase in consumption measures (Will et al., 2009). Therefore, the current study investigated whether a sub-threshold dose of the endocannabinoid 2-AG alter the feeding behaviors driven by intra-accumbens DAMGO.

2. Results

2.1. Consumption

An ANOVA conducted on the food consumption during the total 2hr test session revealed no main effect of 2-AG pretreatment (vehicle or 0.25 μg) ($F(1,36)=1.99$, *ns*), a significant main effect of DAMGO treatment (saline, 0.025μg, or 0.0025μg) ($F(2,36)=14.85$, $p < .0001$), and no interaction ($F(2,36)=1.20$, *ns*) (Fig. 2). As can be observed in the smaller inset in Figure 2, there was a strong trend of decreasing consumption across time with the majority of consumption following all treatments occurring in the first 30 minutes. To further analyze this trend and treatment effects across time, an ANOVA examining 2-AG pretreatment×DAMGO treatment×time interval interaction was conducted. There was again no main effect of 2-AG pretreatment ($F(1,144)=1.58$, *ns*), yet a significant main effect of DAMGO treatment ($F(2,144)=12.22$, $p < .0001$), and time interval ($F(3,144)=183.15$, $p < .0001$). The only significant interaction observed was a DAMGO treatment×time interaction ($F(6,144)=12.91$, $p < .0001$). Post hoc comparisons of consumption during the first 30 min interval revealed that the highest dose of DAMGO, with or without 2-AG pretreatment, was significantly increased ($p < .05$) compared to control treatment (vehicle preatreatment + saline treatment). No other treatment comparison reached significance ($p>.05$).

2.2. Food hopper entries

An ANOVA conducted on the total number of food hopper entries during the 2 hr test session revealed no main effect of 2-AG treatment ($F(1,36)=2.12$, *ns*), a significant main effect of DAMGO treatment ($F(2,36)=3.59$, $p < .05$), and a significant 2-AG×DAMGO treatment interaction ($F(2,36)=3.60$, $p < .05$). As displayed in Fig. 3, post-hoc comparisons revealed that intra-accumbens 2-AG and both DAMGO doses did not increase total hopper entries alone ($p > .05$). However, the combined administration of 2-AG and the high

DAMGO dose produced a significant increase in total number of hopper entries compared to both vehicle treatment ($p < 0.001$) or the high DAMGO dose alone ($p < 0.005$). As can be observed in the smaller inset in Figure 3, there was a strong trend toward a decrease in hopper entries across time, with the majority occurring in the first 30 minutes. To further analyze this trend and treatment effects across time, an ANOVA examining 2-AG pretreatment×DAMGO treatment×time interval interaction was conducted. There was no main effect of 2-AG pretreatment ($F(1,144)=3.05$, *ns*), yet a significant main effect of DAMGO treatment ($F(2,144)=6.12$, $p < .005$), and main effect of time interval ($F(3,144)=73.86$, $p < .0001$). However, the only significant interaction observed was a 2-AG×DAMGO interaction ($F(2,144)=7.53$, $p < .001$). No significant interactions of either treatment across time were observed.

2.3. Duration of food hopper entries

An ANOVA conducted on total duration of food hopper entries during the 2 hr test session revealed no main effect of 2-AG treatment ($F(1,36)=.018$, *ns*), a significant main effect DAMGO treatment ($F(2,36)=10.15$, $p < .0005$), and no 2-AG×DAMGO treatment interaction ($F(2,36)=.16$, *ns*) (Fig. 4). As can be observed in the smaller inset in Figure 4, there was a strong trend toward a decrease in food hopper entry duration across time, with the majority occurring in the first 30 minutes. To further analyze this trend and treatment effects across time, an ANOVA examining 2-AG pretreatment×DAMGO treatment×time interval interaction was conducted. There was no main effect of 2-AG pretreatment ($F(1,144)=0.012$, *ns*), yet a significant main effect of DAMGO treatment ($F(2,144)=6.7$, $p < .005$), and time interval ($F(3,144)=111.06$, $p < .0001$). The only significant interaction observed was a DAMGO treatment×time interval interaction ($F(6,144)=12.35$, $p < .0001$). Post hoc comparisons showed that both low ($p < .05$) and high ($p < .0001$) doses of DAMGO produced significantly longer hopper entry durations during the first 30 min interval, compared to vehicle pretreatment×saline treatment. No significant effects of DAMGO treatment in other time intervals were observed.

2.4. Locomotor Activity

An ANOVA conducted on the total horizontal beam breaks during the 2 hr test session revealed 2-AG treatment approached significance ($F(1,36)=3.57$, $p = .066$), while there was a main effect of intra-accumbens DAMGO treatment ($F(2,36)=11.2$, $p < .0002$), and a significant 2-AG×DAMGO treatment interaction ($F(2,36)=4.18$, $p < .05$). As displayed in Fig. 5, post-hoc comparisons revealed that intra-accumbens DAMGO administration produced significant increases in locomotor activity at both low ($p < 0.05$) and high ($p < 0.05$) doses, compared to vehicle treatment. The combined intra-accumbens treatment of 2-AG and the low dose of DAMGO led to similar activity levels as following the low dose of DAMGO by itself, however 2-AG and the high DAMGO dose combined treatment produced a significant increase in locomotor activity above that observed by the high DAMGO dose alone ($p < 0.005$). As can be observed in the smaller inset in Figure 5, there was a trend toward a decrease in locomotor activity across time. To further analyze this trend and treatment effects across time, an ANOVA examining 2-AG pretreatment×DAMGO treatment×time interval interaction was conducted. There was a main effect of 2-AG pretreatment ($F(1,144)=7.95$, $p < .01$), DAMGO treatment

(F(2,144)=24.95, p < .0001), and time interval (F(3,144)=33.45, p < .0001). The only significant interaction observed was 2-AG×DAMGO treatment (F(2,144)=9.3, p < .001). No significant interactions of either treatment across time were observed.

3. Discussion

The present study demonstrated that co-administration of the endocannabinoid 2-AG and μ-opioid receptor agonist DAMGO into the nucleus accumbens led to a potentiating influence on select food-directed approach and locomotor behaviors associated with high-fat feeding. The combined treatment of intra-accumbens 2-AG and DAMGO led to a significant increase in approach and general locomotor activity behavior yet produced only a marginal non-significant trend on increasing consumption. The majority of consumption following all treatments occurred in the first 30 minutes of the 2hr session. While endocannabinoid and opioid treatments have been shown to interact in the context of other behaviors, this is the first study to examine and demonstrate a significant interaction of the endocannabinoid 2-AG and DAMGO on high-fat feeding associated approach and locomotor behaviors.

In confirmation of previous reports, intra-accumbens DAMGO administration alone produced a significant increase in high-fat consumption at both the low and high doses used. These particular DAMGO doses were chosen as they represent the middle of the dose-response range shown to increase consumption of high-fat diet, representing doses near or just above threshold, and below the dose that produces maximal consumption (Zhang et al., 1998). In regard to 2-AG, a previous study examining chow intake, demonstrated that a 0.125μg dose of 2-AG was subthreshold, and a higher 0.5μg dose produced the maximal response (Kirkham et al., 2002; Deshmukh & Sharma, 2012). Therefore, we chose a 2-AG dose (0.25μg) that represented the middle of this range to examine its interaction with DAMGO, predicting it to have a marginal effect on consumption. However, the inability of combined administration of 2-AG and DAMGO to increase feeding above either treatment by itself could reflect differences in experimental conditions. Two of these differences were time of testing or diet, as Kirkham and colleagues (2002) assessed chow feeding during the onset of the dark cycle. This may suggest that homeostatic feeding processes are more sensitive to the influence of 2-AG than those modeled by DAMGO high-fat procedure (Baldo et al., 2013 for review), yet further study would be necessary to characterize.

The dose of 2-AG used in the current study was subthreshold by itself for all food-directed behaviors and locomotor activity assessed, yet exaggerated specific behavioral measures following co-administration of DAMGO. These effects were observed through the use of additional measures, including food hopper approach behaviors and locomotor activity. Approach behaviors included the number and duration of feeding bouts, as defined by total number and duration of beam breaks near the front entry of the food hopper over the 2hr feeding session. The combined administration of 2-AG and the high dose of DAMGO led to a 2-fold increase, compared to control treatment levels. The increase was most evident in the first 60 min of the 2hr test session then decreased to control levels, possibly through an influence of satiety factors. The total time of food hopper entry duration was significantly increased above control levels following the high DAMGO dose treatment, yet 2-AG treatment by itself or in combination with DAMGO had no effect on this measure.

While intra-accumbens administration of 2-AG has been shown to produce increased intake of palatable chow (Kirkham et al., 2002) and high-fat or high-carb diets (Deshmukh & Sharma, 2012), its interaction with ventral striatal opioids had not been explored. The current study targeted a region of the nucleus accumbens that DAMGO administration produces the largest consumption increase of a high-fat diet (Zhang & Kelley, 2000). CB1 and μ-opioid receptors have been shown to be co-localized on axons and dendrites (Pickel et al., 2004) and exhibit a functional interaction (Manzoni et al., 2001) within this region. A potentiated response was observed between opioid and cannabinoid systems on feeding using antagonists (Tallet et al., 2009), yet few studies have explored a similar response produced by activation of these two systems with agonists. One such study demonstrated that administration of the CB1 agonist WIN55212-2 and DAMGO into the medial shell of the accumbens led to an exaggerated intake of a palatable diet in a similar manner (Skelly et al., 2010). In contrast to the present findings, these authors did not observe an interaction on ambulation or approach behaviors, suggesting the pharmacological actions of the endocannabinoid 2-AG within the accumbens may be different compared to certain selective CB1 agonists. It may also be related to site of action, as Skelly and colleagues (2010) targeted the medial shell, where DAMGO effects on consumption of high-fat are less pronounced than those observed following administration into the core and lateral shell border (Zhang & Kelley, 2000). To our knowledge, the actions of 2-AG within the medial shell on appetitive approach or locomotor behaviors associated with a high-fat diet have not been examined. Future studies examining the other endogenous cannabinoid anandamide (Soria-Gómez et al., 2007), as well as the effect of cannabinoid receptor antagonists to assess the specificity of 2-AG acting on cannabinoid receptors, would aid in interpretation of the current findings. A recent review also raises the implication of endocannabinoids, 2-AG and anandamide, capable of having influences on behavior through the actions of their metabolites (Silvestri and Di Marzo, 2013).

In any model of feeding, an examination of all associated behaviors contributing to both the appetitive food-directed approach (i.e. hopper entries) and consummatory (i.e. consumption) phases of feeding has been proven critical to understanding the complex nature of this behavior. Examination of the neural circuitry of these two phases of feeding has demonstrated distinct circuits that mediate each, yet are both important for driving behaviors associated with food reinforcement (Will et al., 2009; Petrovich and Gallagher, 2003). In addition to the benefit of providing insight into each phase of feeding, it is critical to consider how changes in multiple behaviors expressed during feeding could influence the other. In the current study, the observed changes in locomotor activity could be interpreted to be a non-specific response or an anticipatory/appetitive behavior associated with and directed towards the food. A non-specific increase in locomotor activity could compete with and diminish consumption by decreasing sustained contact with food, yet the combined treatment of 2-AG and the high dose of DAMGO led to the highest level of both locomotor activity and consumption in the current study. This trend has been observed previously, as intra-accumbens DAMGO increased locomotor activity during a sucrose-drinking task and this increased activity did not interfere with the parallel increase in total sucrose intake (Zhang and Kelley, 1997).

In conclusion, the present findings provide a novel characterization of an opioid-cannabinoid interaction within the nucleus accumbens and its resulting influence on select behaviors within a model of high-fat feeding. They confirm that intra-accumbens administration of a subthreshold dose of the endogenous cannabinoid 2-AG increases DAMGO-induced approach and locomotor behaviors associated with high-fat feeding.

4. Experimental Procedure

4.1. Subjects

Subjects were 7 male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250 to 300 g. Rats were housed in Plexiglas cages, 2–3 per cage throughout the entire experiment, in a temperature and humidity controlled room at 22 °C and maintained on a 12/12 light:dark cycle (lights on at 0700hr) with all experiments being conducted during the light phase (1100–1400hr). Throughout the experiment, animals were allowed unrestricted access to water and standard laboratory chow (Purina LabDiets, St. Louis, MO) in their home cages. Experimental procedures used were in accordance with the University of Missouri Institutional Animal Care and Use Committee guidelines and approved protocols.

4.2. Surgical placement of cannulae

Animals were anesthetized with a ketamine/xylazine mixture of 90 mg/kg ketamine/9 mg/kg xylazine (Sigma, St. Louis, MO) and stereotaxically implanted with bilateral guide cannulae (23 gauge, 10 mm) aimed at the nucleus accumbens using the following coordinates, from bregma: +1.4 AP, ±2.0 ML, −7.8 DV (Paxinos and Watson, 1998). Following standard flat skull procedures, the guide cannulae were secured to the skull using stainless steel screws and jet acrylic (Lang Dental Mfg. Co. Inc., Wheeling, IL). Following surgeries and throughout experiments, wire stylets (10.5 mm) were kept in guide cannulae to prevent blockage. Animals were allowed one week for recovery prior to treatment.

4.3. Drugs and microinjection procedure

The μ-opioid receptor agonist $_{D}$-Ala^{2},N,Me-Phe^{4},Gly-ol^{5}-enkaphalin (DAMGO) (Sigma Chemical Company, St. Louis, MO) was dissolved in sterile 0.9% saline and the cannabinoid receptor agonist 2-arachidonylglycerol (2-AG) (Sigma Chemical Company, St. Louis, MO) was dissolved in 10% DMSO. Animals were gently hand-held during the injection procedure. Infusions were administered using a microdrive pump (Harvard Apparatus, South Natick, MA) connected via polyethylene tubing (PE-10). After the stylets were removed, the drug or vehicle was infused through 12.5-mm 33 gauge injector cannulae, thus allowing the injector tips to extend 2.5 mm beyond the end of the 10 mm guide cannulae. The rate of injection was equated to produce an injection volume of 0.50μl for both 2-AG and DAMGO. The dose of 2-AG was 0.25μg/side and either 0.025μg or 0.0025μg/side of DAMGO over a 93 second duration. Injectors were removed and stylets replaced following infusion.

4.4. Specialized Diet

The high fat diet (HFD) was obtained from Teklad Diets (Madison, WI) and contained 278.3 g/kg vitamin free casein, 4.2 g/kg DL-methionine, 100.0 g/kg sucrose, 441.2 g/kg

hydrogenated vegetable shortening, 77.7 g/kg linoleic safflower oil, 26.3 g/kg cellulose, 53.3 g/kg AIN-76 mineral mix, 15.2 g/kg AIN-76A vitamin mix, and 3.8 g/kg choline chloride. The diet consisted of 6.2 kilocalories/gram; 16.5% kcal from protein, 7.8% kcal from carbohydrates, and 75.6% of kcal from fat.

4.5. Apparatus and behavioral assessment of feeding behavior

Testing took place in a room separate from the colony room in eight Plexiglas (30.5 cm×24.1cm×21.0 cm) feeding chambers (Med Associates, St. Albans, VT). Feeding chambers were equipped with four infra-red photo-beams at intervals of 6 cm and positioned 4.3 cm above the bar floor to measure feeding associated locomotor activity across the chamber, an automated weigh scale for the food hopper to continuously monitor the weight of the hopper while automatically correcting for spillage, and a water bottle. The feeding hopper and water bottle were located on opposite corners of the same side of the chamber wall and a removable waste tray was located beneath the bar floor. Measurements were calculated based on the entire 2hr test session. These included locomotor activity (number of horizontal beam breaks), amount consumed (grams of diet consumed), hopper entries (number of times beam at entry point of recessed food hopper was broken), hopper entry duration (total duration beam at entry point of recessed food hopper was broken), duration per entry (hopper entry duration divided by number of hopper entries). Manual weights of the high fat diet were taken at the end of the session in addition to the automated measurements by the software to ensure accuracy. These two measures were very similar (i.e. differences ranging between only 0 – 0.4 grams for the full 2hr measurement), therefore, all data represents the automated measures. Measurements were calculated by monitoring software, Med-PC Version IV (Med Associates, St. Albans, VT).

4.6. General procedure timeline

Animals had *ad libitum* access to water and high fat diet (approximately 35 g) in the feeding chambers during all testing sessions. Subjects were placed in the feeding chambers for 2hr daily for 6 days. During the last 2 days of this habituation phase, animals were acclimated to the injection procedure. On Day 5, a 10.0 mm injector was inserted and left in place for 2 min, though no volume was administered. On Day 6, animals received an injection of saline into the accumbens with a 12.5 mm injector. Animals then received drug and vehicle treatments in a within-subjects, counter-balanced design. Immediately following each drug treatment, the animal was placed in the feeding chamber for 2hr of individual automated behavioral monitoring. At the end of the 2hr session, animals were returned to their home cages and returned to the colony room. One day separated treatment sessions.

4.7. Histology

At the conclusion of the experiment, animals were overdosed with sodium pentobarbital and perfused transcardially using heparinized saline (200 ml) followed by 10% buffered formalin solution (200 ml). Brains were extracted and kept in 20% sucrose and 10% formalin mixture. Frozen serial sections (in 40 μm slices) of the injection site were collected and mounted on slides, stained with cresyl violet and cover slipped. Cannulae placements of all animals were assessed with a light microscopy for proper placement. No animals were

excluded based on criteria of injector placement. A representative photomicrograph and schematic representing the tip of the injector track for all rats is represented in Fig. 1.

4.8. Statistical Analysis

Data was analyzed using a 2 (2hr total) or 3-way (30 min intervals) ANOVA, followed by posthoc orthogonal contrasts of means when appropriate.

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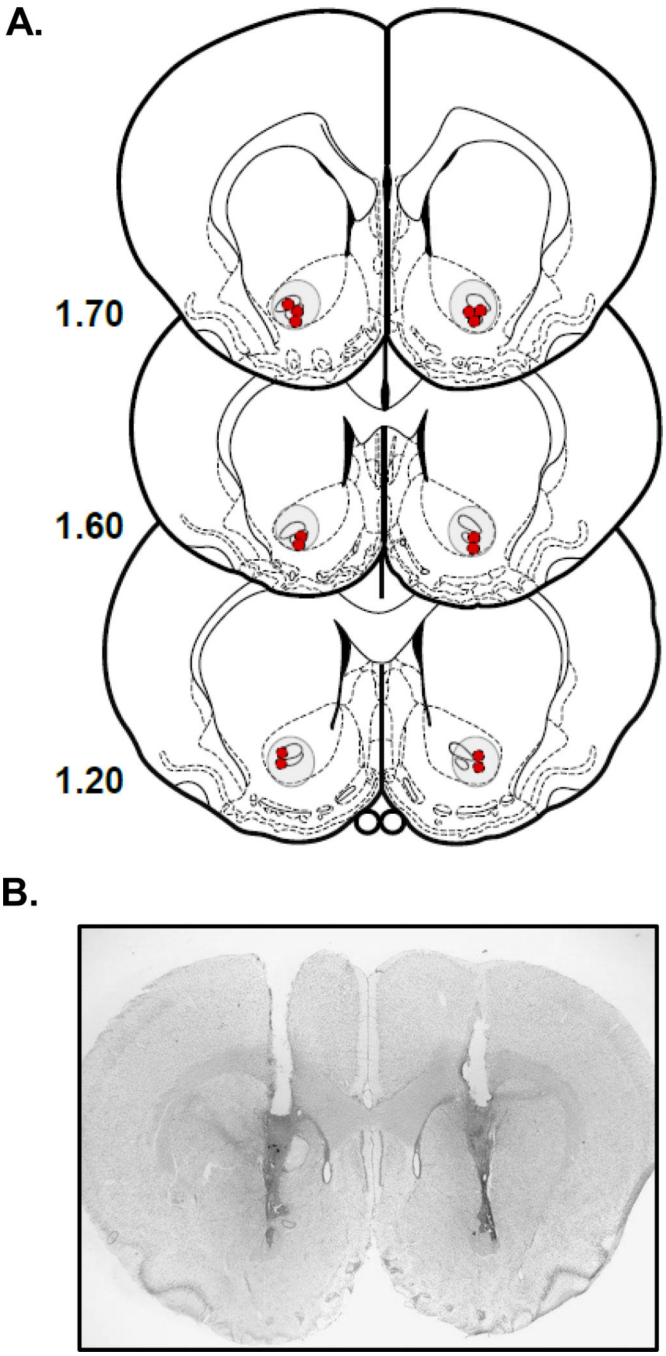

Fig. 1.

A) Bilateral locations of the most ventral track left by each injector for each rat. Number to left of each figure is distance (mm) anterior to bregma. (modified from Paxinos and Watson, 1998). **(B)** Photomicrograph depicting representative placement of bilateral cannulae and injector track.

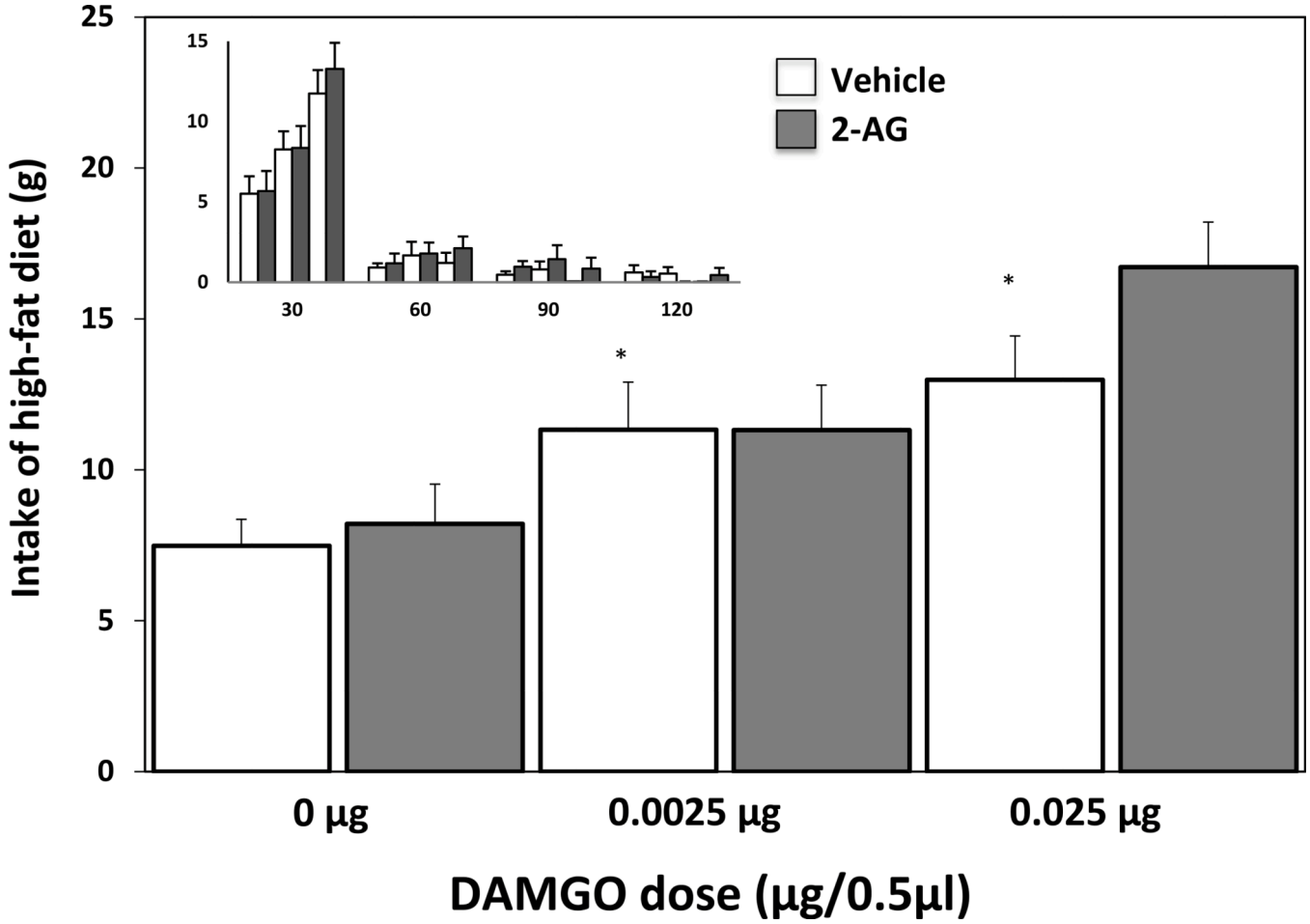

Fig. 2.

Influence of intra-accumbens 2-AG (0.25µg/0.5µl) and DAMGO (0.0025µg or 0.025µg/0.5µl) on consumption levels (* = $p < .05$; compared to Vehicle – 0µg DAMGO coadministration). The inset graph depicts the same data across 30 min intervals; the x-axis represents 30-min time intervals across the 2hr testing period (30, 60, 90, and 120 min) and the y-axis represents the grams of high-fat consumed.

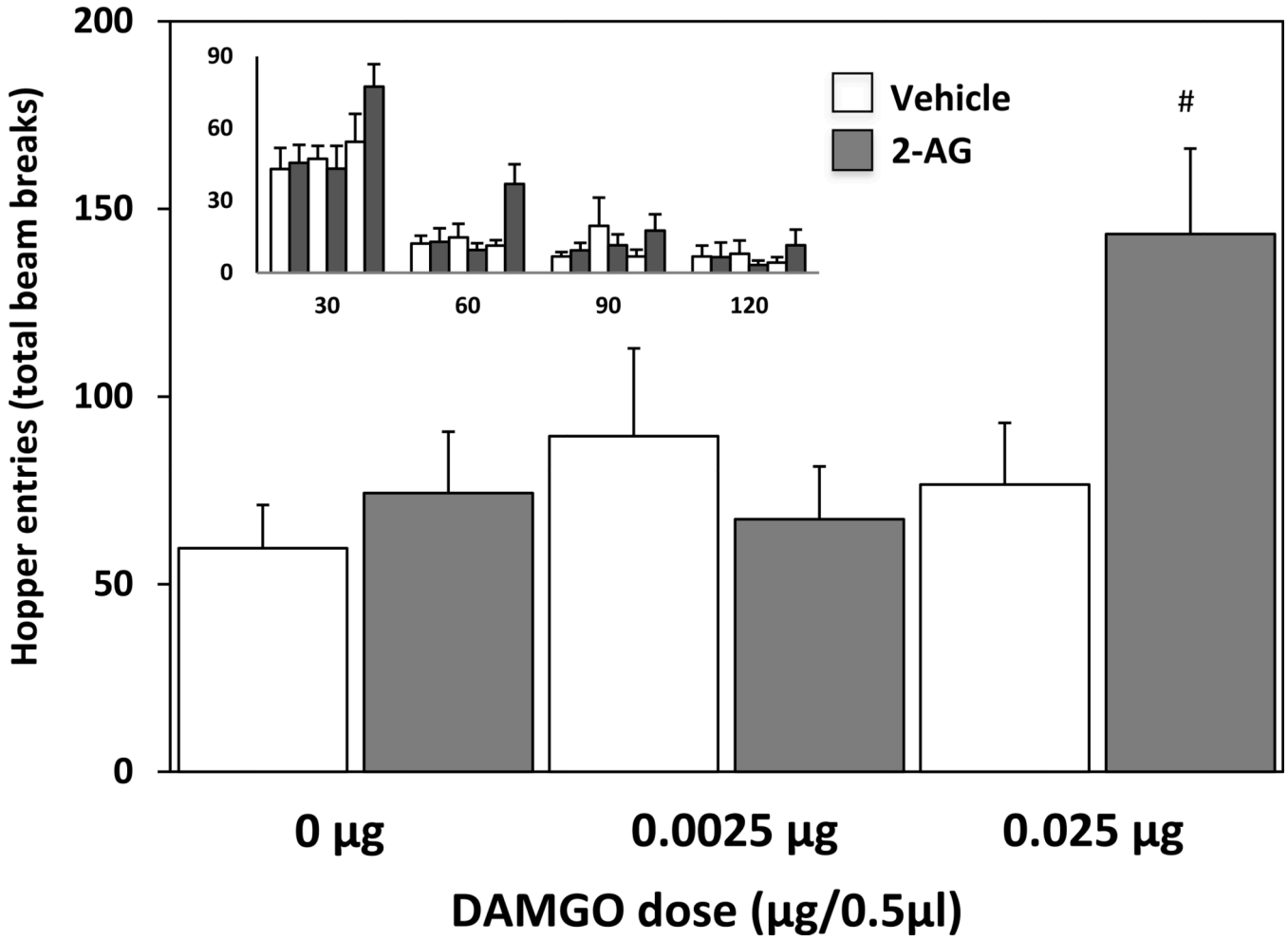

Fig. 3.

Influence of intra-accumbens 2-AG (0.25µg/0.5µl) and DAMGO (0.0025µg or 0.025µg/0.5µl) on food hopper entries (# = $p < .05$; compared to both Vehicle – 0µg DAMGO and Vehicle - 0.025µg DAMGO co-administration). The inset graph depicts the same data across 30 min intervals; the x-axis represents 30-min time intervals across the 2hr testing period (30, 60, 90, and 120 min) and the y-axis represents the total number of food hopper entries.

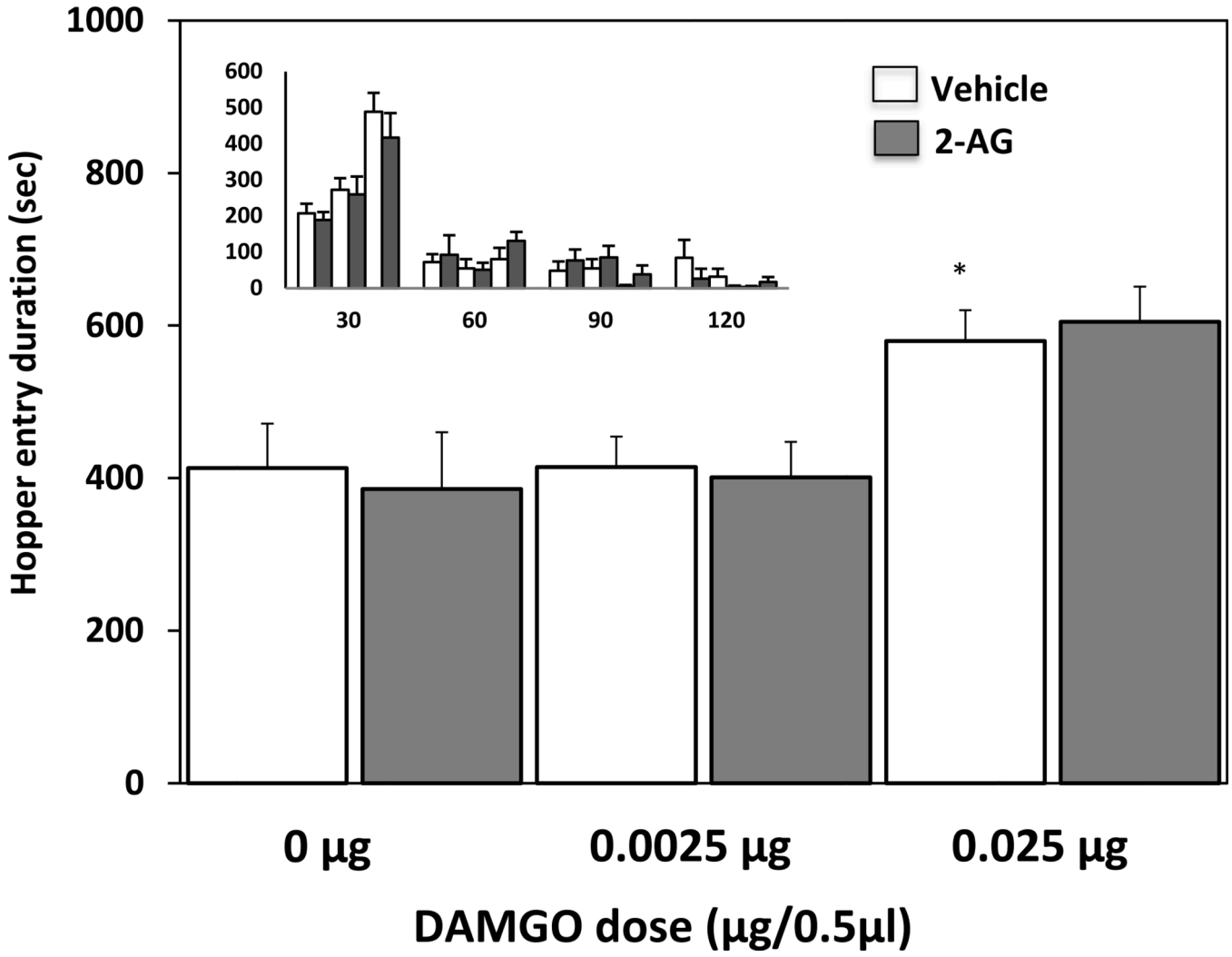

Fig. 4.

Influence of intra-accumbens 2-AG (0.25µg/0.5µl) and DAMGO (0.0025µg or 0.025µg/0.5µl) on total food hopper entry duration (* = p <.05; compared to Vehicle – 0µg DAMGO co-administration). The inset graph depicts the same data across 30 min intervals; the x-axis represents 30-min time intervals across the 2hr testing period (30, 60, 90, and 120 min) and the y-axis represents the total duration of food hopper entries.

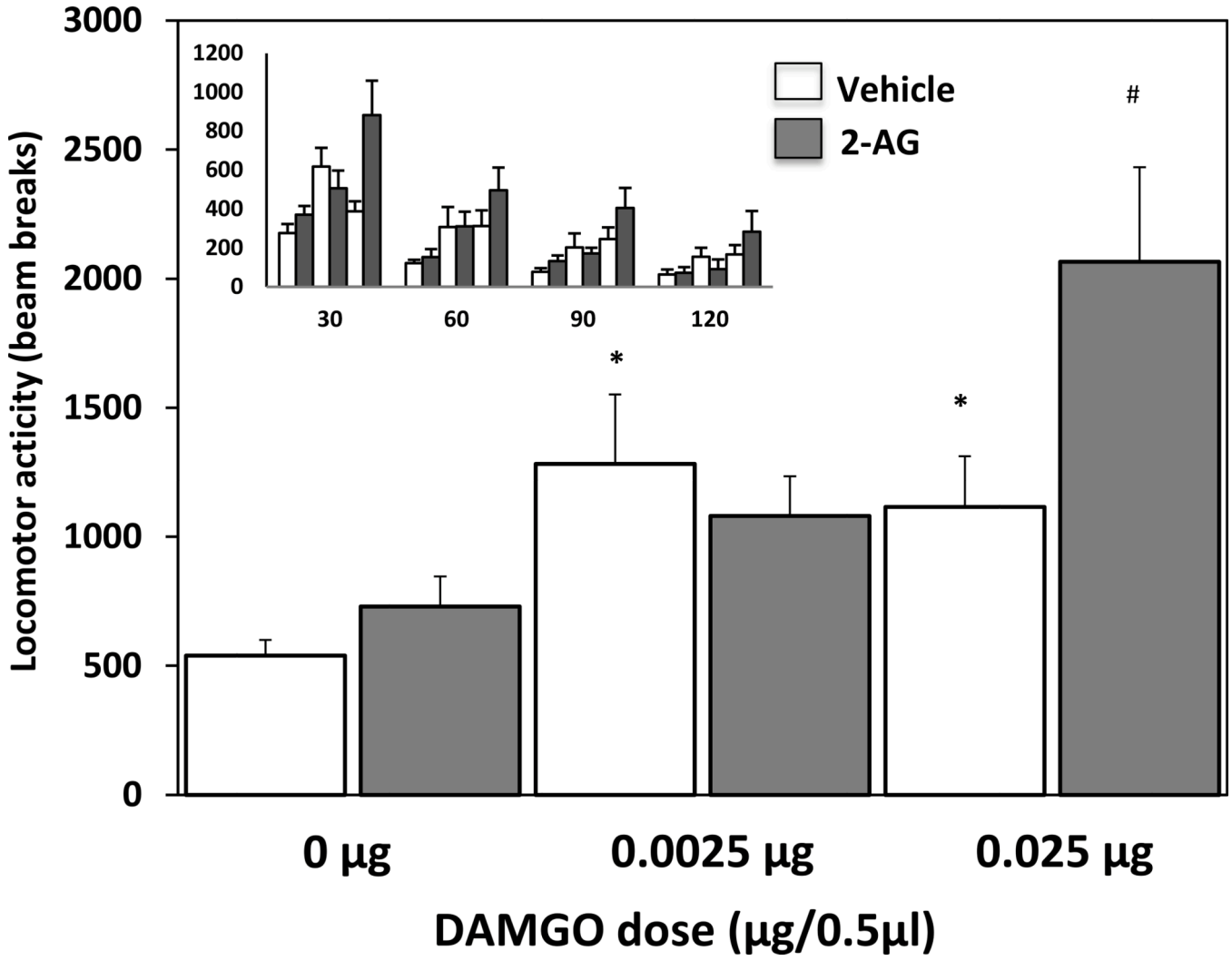

Fig. 5.

Influence of intra-accumbens 2-AG and DAMGO on locomotor activity (# = $p < .05$; compared to Vehicle - 0.025µg DAMGO co-administration) (* = $p < .05$; compared to Vehicle – 0µg DAMGO co-administration). The inset graph depicts the same data across 30 min intervals; the x-axis represents 30-min time intervals across the 2hr testing period (30, 60, 90, and 120 min) and the y-axis represents the locomotor activity (beam breaks).