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The GLP-1 agonist exendin-4 attenuates self-administration of sweetened fat on fixed and progressive ratio schedules of reinforcement

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Abstract

GLP-1 agonists such as exendin-4 (EX4) are used in the treatment of type 2 diabetes and have the additional benefit of promoting weight loss. GLP-1 agonists decrease feeding through peripheral affects, but recent evidence suggests they may also influence sweet or high fat preference, as well as the motivation to obtain these tastants. Yet it remains unclear how GLP-1 induced alterations in food preference influences the decrease in overall feeding. The current study sought to determine if EX4 affects the reinforcing strength and consumption of a highly palatable sweet/fat reinforcer. Rats were trained to self-administer sweetened vegetable shortening (SVS) under fixed (FR) and progressive ratio (PR) schedules of reinforcement. EX4 (0.3 - 2.4 μg/kg, IP) administered one hour prior to operant sessions significantly reduced responding for SVS under both FR and PR schedules (e.g. total reinforcers and breakpoints, respectively), although the lowest active dose (0.6 μg/kg) significantly suppressed FR responding only. EX4 also dose dependently decreased locomotor activity (0.6-2.4 μg/kg doses), but did not enhance acute kaolin intake, indicating that EX4-induced nausea did not influence the self-administration results. Analysis of ED_{50} values show that EX4 is more effective at inhibiting FR responding versus PR, indicating that EX4 may have more potent effects on consummatory versus appetitive feeding behaviors. Although EX4 caused generalized behavioral suppression, these results cannot fully explain the decreases in operant responding. For example, the 0.6 μg/kg dose inhibited only FR responding, even though the rats were physically capable of responding at a higher rate during PR sessions. In addition, the rate of intake was constant at the beginning of the sessions in both PR and FR schedules, regardless of the dose. Together these data suggest that EX4 inhibits consumption of a palatable high sweet/high fat reinforcer potentially through altering satiety.

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1. Introduction

Synthetic GLP-1 peptide agonists such as exendin-4 (EX4) are used for the treatment of type 2 diabetes mellitus (T2DM) primarily due to their ability to induce insulin secretion and normalize glucose levels through peripheral mechanisms. However, mounting evidence suggests that GLP-1 agonists are also effective at regulating appetite, food intake, and eating preferences. EX4 is often prescribed off-label for the treatment of obesity due to its ability to decrease food intake and promote satiety (Flint et al., 1998). Peripheral administration of EX4 has also been shown to produce dose-dependent reductions in meal size (Scott & Moran, 2007). The suppression of food intake lends itself well to the use of these drugs in the management of T2DM; a disorder characterized not only by uncontrolled blood glucose levels, but also heavily influenced by body weight and obesity.

Peripheral GLP-1 is produced by L-cells in the gastrointestinal tract (Hayes, 2010) and is released in direct proportion to meal size to regulate glycemia by enhancing glucose utilization by peripheral tissues and delaying gastric emptying time (Barerra et al., 2001). In addition to these powerful peripheral effects, it has been well established that GLP-1 profoundly impacts CNS function (Holst, 2007). GLP-1 is produced centrally by neurons in the nucleus tractus solitarius (NTS) of the dorsal medial medulla (Merchanthaler et al., 1999). The NTS receives afferent vagal input from the gastrointestinal tract and is activated by gastric distention (Vrang et al. 2003). GLP-1 activity in the NTS appears to be important in the regulation of normal food intake and the promotion of satiety (Hayes et al., 2009). GLP-1 receptors (GLP-1R) in the brainstem and hypothalamus help regulate glycemia and the suppression of food intake, respectively (Schick et al., 2003). Thus, central GLP-1 signals the end of a meal when energy needs have been met through homeostatic feedback mechanisms.

Mounting evidence suggests that GLP-1 signaling in reinforcement pathways may contribute to the reduction in food intake induced by GLP-1 and EX4 by altering the reinforcing efficacy of different types of foods. GLP-1 neurons in the NTS project to mesolimbic regions including the ventral tegmental area (VTA) and nucleus accumbens (NAc), which contain GLP-1R (Aldaheff et al., 2012). Theoretically, GLP-1 signaling could decrease the motivational value of food when the animal is sated. In support of this, recent studies have demonstrated that microinjections of EX4 into the VTA decrease food intake and body weight when animals are provided access to a variety of energy sources, including high fat, high sugar and standard rat chow diets (Yang et al. 2014, Dickson et al. 2012). Notably, it has been demonstrated that EX4 microinfusions into the VTA decreases responding for sucrose under a progressive ratio schedule of reinforcement and decreases conditioned place preference for chocolate pellets (Dickson et al., 2012). In addition, it appears that VTA EX4 microinfusions selectively reduced high fat food intake for 24 hours while enhancing standard chow intake in rats given ad libitum access to both standard and high fat chow (Aldaheff, et al., 2012). Thus, GLP-1 signaling in the VTA appears to directly suppress the reward value of some highly palatable and energy dense foods, and may shift food preference away from energy dense foods.

While EX4 can modulate both satiety and reinforcement pathways, it is not known whether the reduction in total food intake and potential shift in preference to less energy dense foods are due to enhancing satiety signals as the animal's energy needs are met or by decreasing the rewarding salience of high fat foods. Most studies to date have used palatable but relatively low energy dense foods in operant models of food-seeking behaviors. To better understand how EX4 regulates the motivation for highly energy dense palatable foods, we have elected to study the self-administration of a palatable fat based food source, vegetable shortening sweetened with sucrose. Rats maintained under standard free feed conditions were trained to self-administer sweetened vegetable shortening (SVS) and challenged with pharmacologically relevant doses of EX4 prior to the operant sessions. Fixed ratio (FR) and progressive ratio (PR) schedules were both examined to help model feeding patterns that engage satiety and reinforcement processes. Specifically, FR responding results in high levels of SVS intake quickly within the session while PR responding results in much lower levels of intake over a longer time period. In addition, since many T2DM patients reportedly suspend their GLP-1 agonist treatment due to nausea and malaise, we have examined dose-dependent changes in both pica response and locomotor activity to ensure that changes in feeding are not due to nausea during the session or an overall behavioral suppression. Together these experiments provide insight into how EX4 modulates motivation and satiety for fat based foods, and may help explain the potential shift in preference away from a high fat diet (Aldaheff, 2012).

2 Methods

2.1 Subjects

Twelve male Sprague Dawley rats weighing between 350-375 g were purchased from Harlan laboratories (Indianapolis, IN) and individually housed under a 12:12 light-dark cycle, with lights on at 3:00 PM. Food and water were available ad libitum except when otherwise reported. Rats were weighed weekly. All procedures were reviewed and approved by the UB Institutional Animal care and Use Committee.

2.2 Intermittent Access Procedure and Operant Training

Rats were exposed to an intermittent access procedure to ensure high levels of intake during self-administration (Corwin, 2004). Rats were food restricted for a single 24-hour period. After food restriction, a 3 inch diameter jar containing 10g of the sweetened vegetable shortening (SVS; 10% sucrose whipped in vegetable shortening (Crisco™) was introduced into the home cage for one hour. Rats were given access to the SVS every Monday, Wednesday, and Friday for five weeks. The total amount consumed in one hour was recorded for each subject. After five weeks of SVS intermittent access, the rats began training in the operant task. Rats were again food restricted for a single 24-hour period before the start of the first acquisition training session. Rats only had access to the SVS during operant sessions, and were allowed chow ad libitum following the session. All operant sessions occurred in individual custom-built 33 cm × 33 cm × 33 cm operant chambers (Faircloth, Winston-Salem, NC). Each chamber was equipped with an active lever attached to a pump housing a 20-ml glass syringe loaded with the SVS mixture. Each pump was calibrated to deliver 0.1g of SVS per reinforcer followed by a 20 second time out

period. SVS was delivered from the glass syringe onto a flat metal surface affixed to the inside of the operant chamber.

For these experiments we used a custom self-administration software platform, Ghost; which was developed for data collection and analysis using the graphical programming language, LabVIEW (National Instruments, Austin, TX). The required hardware consists of a single NI PCI 6221 multifunction data acquisition card (National Instruments), related cabling, a locally manufactured interface box, and a Microsoft Windows©-based computer. The software program contains several features that were used in this publication as well as many other features that we use regularly. The acquisition portion of the software contains settings to enable the performance of self-administration on a variety of schedules of reinforcement including the fixed and progressive ratio used herein. Additionally, the software contains integrated routines that allow for on-the-fly designing of custom schedules with variable response requirements, cue-presentations, inter-trial durations, and reward delivery parameters. These features are integrated with digital event generation/registration such that TTL pulse information can be shared to synchronize self-administration events with external devices. The analysis portion of the software contains many basic tools for data representation and analysis, figure creation, and exportation to third-party database and spreadsheet software.

One-hour operant sessions ran every day at 10:00AM and 11:15AM. Rats were brought into the procedure room 15 minutes prior to the start of the session to acclimate to the test environment. Initial operant training to acquire the food self-administration procedure consisted of one hour fixed ratio-1 (FR1) sessions, where each press of the lever resulted in dispensing 0.1 g of SVS followed by a 20 sec time out. A maximum of 20 reinforcers were allowed in the training sessions. Rats were considered to have acquired the operant behavior when this maximum number of reinforcers was obtained for five consecutive sessions. Following successful acquisition of the FR task, rats were switched to a progressive ratio schedule in which the response requirement for each consecutive infusion increased according to a set progression of 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603 (Richardson and Roberts, 1996). Breakpoints were recorded and defined as the number of reinforcers earned within a session. Final ratios are the number of responses needed to obtain the final reinforcer.

2.3 Reagents

EX4 was acquired from American Peptide Company (Sunnyvale, CA), and was resuspended in 0.9% saline and stock solutions stored at −80 °C until the day of the experiment. On experimental days EX4 stocks were thawed on ice and diluted with saline to 0.3, 0.6, 1.2 and 2.4 μg/kg. Cyclophosphamide monohydrate was purchased from Sigma and resuspended in saline. SVS consisted of a mixture of Crisco™ All Vegetable Shortening and 10% sucrose (w/v) made by whipping the sucrose into the shortening with a hand mixer until the desired consistency was reached. The SVS was piped into a 60 ml plastic syringe using a bakers piping bag. The 60 ml syringe was then used to load individual 20 ml glass syringes for the self-administration experiments. The consistency of the SVS was set such that 0.1 g of SVS could be administered over approximately 3.5 seconds. Kaolin (hydrated aluminum silicate,

Cat. No. K2-500) and gum arabic (Cat. No. AC25885-2500) were purchased from Fisher Scientific and mixed together at a 99:1 ratio until completely homogenized. 50 ml of distilled water was added per 100 g of the mixture to form a paste roughly the consistency of Play-Doh™. The paste was rolled by hand into a cylindrical shape, and allowed to dry completely for 72 hours at room temperature. A razor blade was used to score indentations along the cylinder approximately every one inch. This allowed us to snap off a pellet roughly the size and shape of the standard rat chow pellets.

2.4 Acute pica testing

After the completion of SVS self-administration experiments a modified kaolin test for pica in response to EX4 was performed. Rats were habituated to the kaolin pellets in their home cage for three days prior to EX4 dosing. During this time kaolin pellets contained in a hanging jar were available within the home cage 24 hours a day. On EX4 test days the rats were injected with saline, 0.6μg/kg, 1.2μg/kg or 2.4μg/kg EX4. At least three days were allowed between tests and the dosing occurred in a balanced Latin square design. One hour after dosing, the rats were introduced to a clean cage with no bedding. Water and standard rat chow was available ad libitum and 8 g of kaolin pellets were available in a separate food hopper. Rats had access to the kaolin for one hour, to mirror the length of the SVS self-administration session. All kaolin was retrieved from the cage, including any shards due to spillage and weighed to determine consumption. Any urination was noted, as this could potentially obscure the kaolin spillage. In general urination was minimal and did not appear to interfere with the final measurement.

2.5 Experimental Design

After acquisition training, rats were maintained on PR for 14 days to determine stable baselines. EX4 was administered in a Latin square design over four sessions such that rats received saline, 0.3, 0.6, 1.2 or 2.4 μg/kg exendin-4 intraperitoneally (i.p.) one hour prior to the start of the operant session. Upon completion of the one-hour self-administration session, the rats were placed in an open field chamber (Med-Associates, ENV-515) where distance travelled was recorded for one hour in 12 five-minute bins. Rats were given at least three days between EX4 trials, during which they were allowed to self-administer SVS under a PR schedule as normal. The kaolin tests for pica began one week after all self-administration sessions were complete. Again a Latin square design was used with at least three days between doses.

2.6 Data analysis

All data were collected and analyzed using Graphpad Prism 6.0. Results were analyzed by one way repeated measures ANOVA with Dunnet's post-hoc test. For calculation of ED50 values, the BP and total reinforcers obtained in PR and FR experiments were converted to % inhibition values based on the average BP or total reinforcers, respectively, obtained with vehicle. The ED50 values were calculated based upon least squares linear regression followed by calculation of 95% confidence limits (Bliss, 1967). Kaolin effects were examined using Student's t–test.

3. Results

3.1 Effects of EX4 on responding under fixed ratio 1 and progressive ratio responding

Rats were maintained on PR sessions for the majority of this study, which minimized the total amount of reinforcer each animal received on a daily basis. Figure 1 demonstrates responding for the entire cohort across the two weeks of daily PR sessions prior to EX4 testing (Figure 1). The mean BP ± SEM for this 14 day period was 8.73 ± 0.40. Please note that the breakpoint is also the final number of individual reinforcers (0.1 g SVS) obtained in the session.

On test days the rats were injected with EX4 or saline one hour prior to beginning SVS administration. Given the relatively long half-life of EX4 we allowed at least three days of recovery between test sessions, during which rats were maintained in standard daily PR sessions. EX4 dose-dependently led to significantly decreased SVS breakpoints (Figure 2A) in PR sessions. Repeated measures one-way ANOVA demonstrates that EX4 produced a significant main effect on both breakpoints [$F(11, 33) = 20.56$, $p < 0.0001$ and final ratio $F(11, 33) = 14.00$, $p = 0.0001$]. Dunnets post-hoc analysis indicates that 2.4 and 1.2 μg/kg EX4 significantly reduced the breakpoint for SVS ($p < 0.05$) but 0.3 and 0.6 μg/kg did not. After converting the BP data to % inhibition of saline levels we calculated an ED_{50} with 95% confidence limits of 1.35 μg/kg (1.16-1.56).

Next, we evaluated responding for SVS under a FR1 schedule of reinforcement (Figure 2B), which allows us to examine the effects of EX4 under conditions that may involve satiety, given that much higher amounts of reinforcer can be obtained under FR1. For example, after saline the average amount of SVS obtained in the PR sessions is 0.8 ± 0.49 g, while 4.58 ± 0.49 g SVS was obtained during FR sessions. In the one-hour self-administration session a theoretical maximum of 180 reinforcers, or 18 g of SVS, could be achieved since the 20-second time allows for only 3 reinforcers per minute. Similar to the PR results, EX4 decreased responding dose-dependently in FR1 sessions from 45.8 ± 4.9 reinforcers for saline to 33.2 ± 4.1, 20.5 ± 3.0, 13.58 ± 2.3, and 6.50 ± 1.55 for 0.3, 0.6, 1.2 and 2.4 μg/kg EX4 doses, respectively (Figure 2B). Repeated measures one-way ANOVA showed that relative to saline, EX4 significantly decreased SVS reinforcers obtained at the 0.6 μg/kg, 1.2 μg/kg and 2.4 μg/kg doses [$F(11, 33) = 30.83$, $p < 0.0001$]. An ED_{50} of 0.62 μg/kg (0.53-0.73) was obtained after conversion to % inhibition of reinforcers obtained under saline.

Event records from representative subjects given saline and EX4 doses are presented in Figure 3 for both PR and FR responding. As seen in Figure 3A, EX4 appears to affect the total number of responses during PR sessions, and not the pattern of responding throughout the session. This is most clearly observed early in the session, where the first few reinforcers are obtained under a relatively easy response requirement (Figure 3B). As the session progressed and the response requirement for each successive reinforcer increased, it appears that EX4 induced premature cessation in responding compared to saline (Figure 3A). Similarly, EX4 did not appear to affect the pattern of responding early in the FR sessions (Figure 3C and D), however two obvious patterns emerged later in the session. Similar to the PR event records, many animals responded by reaching a threshold number of reinforcers

after which responding ceased (Figure 3C), while others appear to reach a threshold relatively early in the session, but initiated new bouts of responding later in the session (Figure 3D). In either case the initial rate of responding appears to be very similar regardless of the doses of EX4 used.

To explore the effects of EX4 on PR responding, we determined the latency to the first lever press in each session (Figure 4). Repeated measures one-way ANOVA established that EX4 did not significantly alter the latency to press the lever [$F(11,33) = 0.7910$ at $p = 0.6473$] between the three different doses of EX4 or saline. However, it is interesting to note that four out of the 12 subjects receiving the highest dose of EX4 waited an average of 2303 seconds to make their first operant response for SVS. Though this increase was not significant, when compared to the average latency of 2.0 seconds seen in the remainder of the groups, these four subjects may represent an important individual difference. The 2.4 μg/kg EX4 appeared to be equally effective in decreasing the total number of reinforcers regardless of the latency.

3.2 EX4 dose dependently inhibits locomotor activity

Previous studies have shown that EX4 and other GLP-1 agonists inhibit locomotor activity. In order to determine if the EX4-induced inhibition of operant responding may be due to an overall decrease in activity, we measured spontaneous activity in an open field for 30 minutes immediately upon completion of the operant session. The mean distance traveled in 30 minutes is presented in Figure 5A, with saline, 0.3, 0.6, 1.2 and 2.4 μg/kg EX4 producing an average of 1491 cm ± 89, 1497 ± 110, 965 ± 118, 713 ± 100, and 265 ± 38, respectively. These data were analyzed by repeated measures one-way ANOVA. There was a significant main effect of dose on locomotor activity $F(10, 40) = 3.566$, $p = 0.0019$. Post hoc analysis revealed that the 0.6, 1.2 and 2.4 μg/kg doses of EX4 significantly differed from saline, but not the 0.3 μg/kg. In Figure 5B we present the time course of locomotor activity in five minute bins. The ED_{50} for percent inhibition of total distance traveled is 1.86 μg/kg (1.41-2.45).

3.7. Rats exhibited normal weight gain throughout the study

Previous studies have established that EX4 can result in weight loss (Hayes et al., 2010). In Figure 6 the rats exhibited weight gain at a normal pace with no noticeable change from standard growth curves (Harlan Laboratories, 2015).

3.8 EX4 does not induce pica during the time of operant behavioral tasks

Larger doses of EX4 (e.g. 3.0, 25 and 75 μg/kg) have been reported to induce nausea (Kanoski, 2001, 2012), which may explain its suppressive effects of EX4 on feeding behaviors. At the end of the operant testing we conducted a pica test to determine if the EX4 would induce nausea in the rats during the operant session. Pica responses are often conducted by measuring cumulative kaolin consumption over several days. However, we were primarily concerned with acute pica responses, which could potentially impact consumption in the operant sessions performed in this study. Previous studies have determined that acute pica responses can be measured shortly after nausea-inducing compounds are administered (Yamamoto et al., 2011). We therefore modified the kaolin test to mirror the timing of the EX4 dosing regimen used in the operant sessions. Rats were first

habituated to kaolin and then injected with saline, 0.6, 1.2 or 2.4 μg/kg EX4 in a Latin square design. One hour later they were placed in a clean cage with a pre-weighed amount of kaolin and total amount of kaolin consumed during the hour was measured. The timing of the kaolin test mirrored precisely the EX4 dosing and operant SVS self-administration sessions, including at least three days between doses. The mean amount of kaolin consumed ± SEM were 0.02 ± 0.01 g, 0.27 ± 0.16 g, 0.29 ± 0.14 g and 0.35 ± 0.20 g for saline, 0.6, 1.2 and 2.4 μg/kg EX4, respectively (Figure 7). The chemotherapeutic agent cyclophosphamide was used as a positive control to ensure that significant kaolin consumption could be induced during the shorter kaolin consumption period. 120 mg/kg of cyclophosphasmide was tested upon completion of the EX4 sessions and the mean ± SEM of kaolin consumed was 1.31 ± 0.30 g. The data were analyzed by repeated-measures one-way ANOVA. There was no significant main effect observed of EX4 on kaolin consumption [$F(11,33) = 2.492$, $p = 0.28$]. Figure 8 illustrates kaolin consumption relative to EX4 and cyclophosphamide. A student t-test demonstrated a significant increase in cyclophosphamide induced kaolin compared to saline controls ($p < 0.05$).

4. Discussion

In this study we sought to define how EX4 treatment affects the consumption and motivation to obtain a highly palatable, high energy fat-based food. This is the first study to our knowledge that has examined the effects of EX4 in an operant model with a fat-based reinforcer. Rats were maintained under free feed conditions throughout the study to keep them in a relatively sated state, where consumption of the SVS is presumably motivated by its palatability and reinforcing efficacy. The break point in the PR schedule is thought to reflect the reinforcing value of a stimulus, and the total number of reinforcers obtained under PR is generally very limited as the response requirement increases through the session. However, under FR1 conditions, where only one response results in a reinforcer, the total number of reinforcers obtained per session can be much higher, and theoretically the animals are limited by physiologic satiety. Consistent with other studies (Dalvi et al., 2012) we found that EX4 suppressed SVS intake in a dose-dependent manner under both schedules at the 1.2 and 2.4 μg/kg doses, however, the 0.6 μg/kg dose suppressed FR responding only. It is interesting to note that 0.6 μg/kg EX4 was less effective at decreasing the PR schedule, which has a higher response requirement. In other words, EX4 significantly decreased FR responding, even though the average number of total responses under the PR schedule was twice that achieved under FR. Therefore it seems unlikely that a generalized locomotor suppression is solely responsible for these results; rather the factor most likely affecting this decrease in FR responding is the high number of reinforcers obtained. These data would indicate that EX4 has more pronounced effects on consummatory rather than appetitive feeding. Figure 8 demonstrates the ED_{50} values obtained throughout this study and clearly indicates a shift between EX4 inhibition of FR and PR responding, as the 95% confidence limits of ED_{50} values do not overlap between these schedules.

Several studies indicate that EX4 differentially effects homeostatic versus hedonic feeding (Aldaheff et al., 2012; Dossat et al., 2001; Richard et al., 2015). It has been suggested that EX4 decreases “food reward” leading to a reduction in operant responses for palatable foods (Dickson et al., 2012). Mounting evidence suggests that GLP-1 agonists decrease reward

salience by altering signaling within mesolimbic areas and may potentially be used as a novel therapeutic to decrease consumption of other hedonic reinforcers including food, drugs and alcohol drinking (Skibicka, 2013). Our data support the notion that GLP-1 agonists attenuate hedonic feeding, and have a higher impact under conditions where satiety more readily factors in.

Alternatively, it has been suggested that the inhibitory effects of EX4 on feeding may be attributed to well-documented side effects including malaise or nausea. For example many patients discontinue GLP-1 agonist treatment due to nausea, vomiting and gastrointestinal distress (Kanoski, 2001). To ensure that the decrease in responding in our operant models was not caused by such confounds we examined the effects of EX4 in a modified kaolin test. Rats readily exhibit a pica response after exposed to substances known to induce nausea in humans. For example, it has been well established that EX4 and other GLP-1 agonists will induce kaolin consumption (Kanoski, 2001, 2012). However these studies have been conducted with relatively long term GLP-1 agonist treatments (days) and with high doses (e.g. 3.0, 25 and 75 μg/kg). For our purposes we sought to determine if EX4 caused acute nausea during the time the rats were undergoing the operant task. We found no significant difference in kaolin consumption with EX4 compared to saline. However, a chemotherapeutic agent, cyclophosphamide, was able to induce a significant increase in kaolin consumption in this acute test, indicating that meaningful results can be observed in the modified test. These results are important for two reasons: first, it indicates that the potential GI distress caused by EX4 does not appear to impact our operant responding results. Secondly, the lack of a pica response with the pharmacologically relevant doses used here is promising for clinical applications of EX4 in the treatment of obesity.

Humans who take GLP agonists such as EX4 often experience therapeutic weight loss, a finding replicated in many other rodent studies where EX4 treatment produced anorectic effects (Barerra et al., 2011; Sisley et al., 2014). However, the rats in our study continued to gain weight normally. Since they received EX4 only on test days it is likely that the low doses tested and long dosing intervals - at least three days - did not support long-term weight loss. In addition, the rats continued to administer SVS on intervening EX4 test days and yet we did not observe any weight gain above the normal weight curves for rats of this age.

GLP-1 agonists have also been shown to decrease locomotor activity, which could potentially confound the results of our operant experiments, particularly in schedules with high response requirements. We observed a decrease in locomotor activity in doses from 0.6-2.4 μg/kg EX4. While this overall suppression in locomotor activity is evident, the animals appeared to respond in a very similar rate at the beginning of the session regardless of dose (Figure 5B). The primary effect appeared to be lowering the ceiling of the total responses for FR and PR schedules (Figure 3A, C), though a subset of rats demonstrated a fragmentation of responding with additional bouts starting later in the session under FR (Figure 3D). Importantly, the inhibitory effects on FR responding occurred even though the subjects apparently had the physical ability to respond (e.g. press the lever more). For example, as figure 3C demonstrates, this subject gave approximately 25 responses at the 0.6 μg/kg EX4 dose during FR responding, but under PR conditions the subject generated 90 lever presses during the 1 hour session, which is even higher than the FR responding after

saline. Thus it appears that the rats are capable of responding at a relatively high rate after lower doses of EX4, and the decrease in responding is presumably due to factors that decrease consummatory behaviors, including potentially increasing satiety levels.

In summary, the GLP-1 agonist, EX4 produced a significant reduction in the intake of sweetened vegetable shortening under both fixed and progressive ratio schedules of responding in animals that were seeking SVS in the absence of hunger. The majority of studies to date have quantified the effects of EX4 on the motivation to obtain palatable foods with relatively low energy density in food deprived animals, or preference for high fat versus standard chow diets. Our study is the first to show that EX4 decreases the motivation to seek out an energy dense fat-based palatable food in the absence of hunger, and this effect appears to more pronounced during conditions where satiety can factor in (e.g. when more is consumed). In order to further understand how GLP-1 agonists impact food intake, future research should attempt to quantify the effects of GLP-1 agonists on food preference in satiated animals, particularly with high energy and palatable foods similar to those consumed by humans,.

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References

Alhadeff AL, Rupprecht LE, Hayes MR. GLP-1 Neurons in the Nucleus of the Solitary Tract Project Directly to the Ventral Tegmental Area and Nucleus Accumbens to Control for Food Intake. Endocrinology. 2011; 153(2):647–658. [PubMed: 22128031]

Barrera JG, Sandoval DA, D'Alessio DA, Seeley RJ. GLP-1 and energy balance: an integrated model of short-term and long-term control. Nature Reviews. Endocrinology. 2011; 7(9):507–516.

Bliss, CI. Statistics in Biology. McGraw-Hill; New York: 1967.

Corwin RL. Binge-type eating induced by limited access in rats does not require energy restriction on the previous day. Appetite. 2004; 42(2):139–142. [PubMed: 15010177]

Dalvi PS, Nazarians-Armavil A, Purser MJ, Belsham DD. Glucagon-Like Peptide-1 Receptor Agonist, Exendin-4, Regulates Feeding-Associated Neuropeptides in Hypothalamic Neurons in Vivo and in Vitro. Endocrinology. 2012; 153(5):2208–2222. [PubMed: 22334721]

Dickson SL, Shirazi RH, Hansson C, Bergquist F, Nissbrandt H, Skibicka KP. The Glucagon-Like Peptide 1 (GLP-1) Analogue, Exendin-4, Decreases the Rewarding Value of Food: A New Role for Mesolimbic GLP-1 Receptors. The Journal of Neuroscience. 2012; 32(14):4812–4820. [PubMed: 22492036]

Dossat AM, Lilly N, Kay K, Williams DL. Glucagon-Like Peptide 1 Receptors in Nucleus Accumbens Affect Food Intake. The Journal of Neuroscience. 2011; 31(41):14453–14457. [PubMed: 21994361]

Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. Journal of Clinical Investigation. 1998; 101(3):515–520. [PubMed: 9449682]

Hayes MR, Bradley L, Grill HJ. Endogenous Hindbrain Glucagon-Like Peptide-1 Receptor Activation Contributes to the Control of Food Intake by Mediating Gastric Satiation Signaling. Endocrinology. 2009; 150(6):2654–2659. [PubMed: 19264875]

Hayes, Matthew R., De Jonghe, Bart C., Kanoski, Scott E. Role of the Glucagon-Like-Peptide-1 Receptor in the Control of Energy Balance. Physiology & behavior. 2010; 100(5):503–10. [PubMed: 20226203]

Hayes MR, Mietlicki-Baase EG, Kanoski SE, De Jonghe BC. Incretins and Amylin: Neuroendocrine Communication Between the Gut, Pancreas, and Brain in Control of Food Intake and Blood Glucose. Annual Review of Nutrition. 2014; 34(1):237–260.

Holst JJ. The Physiology of Glucagon-like Peptide 1. Physiological Reviews. 2007; 87(4):1409–1439. [PubMed: 17928588]

Kanoski SE, Fortin SM, Arnold M, Grill HJ, Hayes MR. Peripheral and Central GLP-1 Receptor Populations Mediate the Anorectic Effects of Peripherally Administered GLP-1 Receptor Agonists, Liraglutide and Exendin-4. Endocrinology. 2011; 152(8):3103–3112. [PubMed: 21693680]

Kanoski SE, Rupprecht LE, Fortin SM, De Jonghe BC, Hayes MR. The role of nausea in food intake and body weight suppression by peripheral GLP-1 receptor agonists, exendin-4 and liraglutide. Neuropharmacology. 2012; 62(5–6):1916–1927. [PubMed: 22227019]

Karen A Scott THM. The GLP-1 agonist exendin-4 reduces food intake in nonhuman primates through changes in meal size. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2007; 293(3):R983–7.

Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. The Journal of Comparative Neurology. 1999; 403(2):261–280. [PubMed: 9886047]

Richard JE, Anderberg RH, Göteson A, Gribble FM, Reimann F, Skibicka KP. Activation of the GLP-1 Receptors in the Nucleus of the Solitary Tract Reduces Food Reward Behavior and Targets the Mesolimbic System. PLoS ONE. 2015; 10(3)

Richardson NR, Roberts DCS. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. Journal of Neuroscience Methods. 1996; 66(1):1–11. [PubMed: 8794935]

Schick RR, Zimmermann JP, Walde T. vorm, Schusdziarra V. Glucagon-like peptide 1-(7–36) amide acts at lateral and medial hypothalamic sites to suppress feeding in rats. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2003; 284(6):R1427–R1435.

Sisley S, Gutierrez-Aguilar R, Scott M, D'Alessio DA, Sandoval DA, Seeley RJ. Neuronal GLP1R mediates liraglutide's anorectic but not glucose-lowering effect. The Journal of Clinical Investigation. 2014; 124(6):2456–2463. [PubMed: 24762441]

Vrang N, Phifer CB, Corkern MM, Berthoud H-R. Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2003; 285(2):R470–R478.

Yamamoto, Kouichi, et al. Time-Course Analysis of Pica in Rats Using an Automatic Feeding Monitoring System. Journal of Pharmacological and Toxicological Methods. 2011; 63(1):30–34. [PubMed: 20451632]

Yang Y, Moghadam AA, Cordner ZA, Liang N-C, Moran TH. Long Term Exendin-4 Treatment Reduces Food Intake and Body Weight and Alters Expression of Brain Homeostatic and Reward Markers. Endocrinology. 2014; 155(9):3473–3483. [PubMed: 24949661]

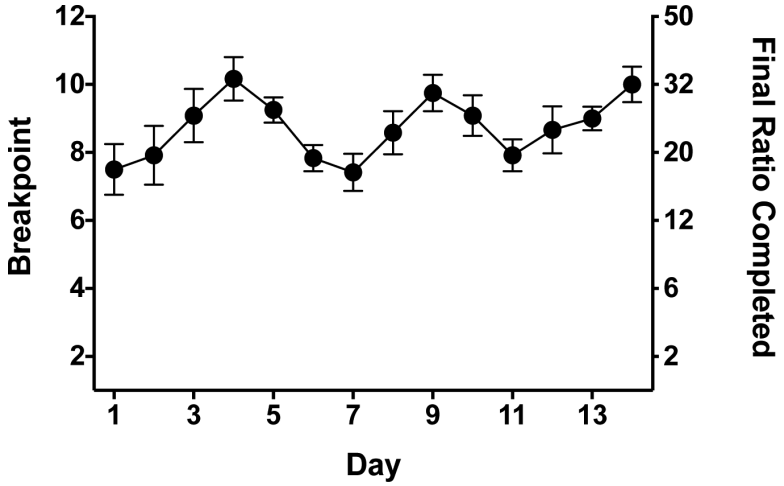

Figure 1. Baseline breakpoints and final ratio for SVS under a PR schedule of reinforcement

BP and final ratios ± SEM were obtained from 14 consecutive daily 1-hour sessions.

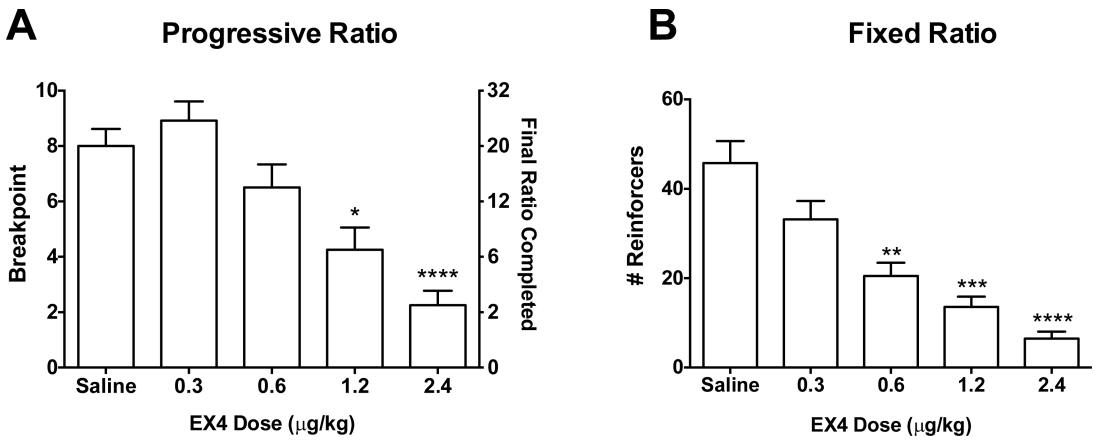

Figure 2. EX4 inhibits SVS responding under PR and FR schedules of reinforcement

In panel A the bars represent the mean breakpoint ± SEM and final ratio obtained in PR sessions, which is also equivalent to the total number of reinforcers obtained in the one hour session. In panel B the mean number of reinforcers ± SEM obtained during FR sessions are presented. Data were analyzed with repeated measures one-way ANOVA and Dunnets post hoc analyses. Significance is designated with * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, p **** ≤ 0.0001.

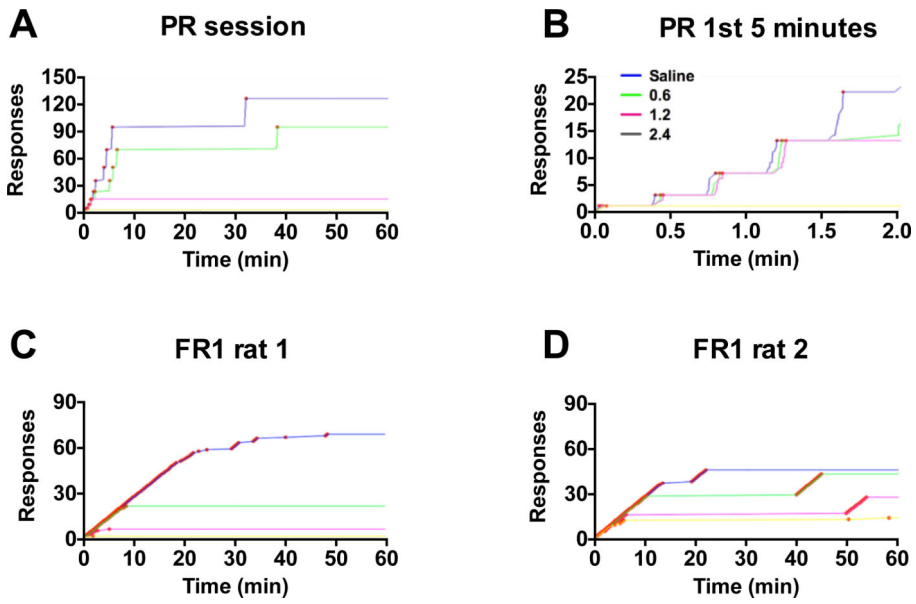

Figure 3. Event records from representative subjects under PR and FR schedules

Panel A shows the cumulative event records during PR responding after EX4 pretreatment from one representative animal. The blue, green, pink and yellow lines represent cumulative lever presses from sessions after saline, 0.6, 1.2 and 2.4 μg/kg pretreatments, respectively. The red dot indicates when 0.1 g of SVS was delivered. Panel B shows the first two minutes of responding from the sessions shown in A. Panel C and D show are representative event records demonstrating the two patterns generally observed after EX4 treatment, in which responding ended prematurely (panel C) or the responding was fragmented during the session. In both PR and FR event records the 0.3 μg/kg dose has not been shown to increase clarity of the figures, as this dose did not significantly alter responding and the cumulative even records were very similar to saline.

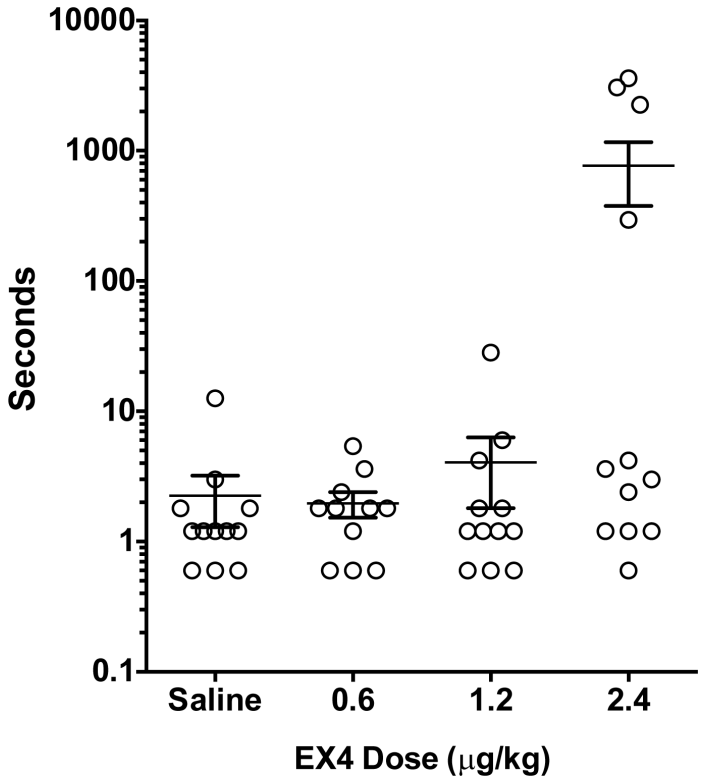

Figure 4. EX4 does not alter latency for first lever press

The horizontal bar represents the average latency in seconds ± SEM until the first lever response for the group in FR sessions. Circles represent data points for individual subjects. Data were analyzed with repeated measures one-way ANOVA and Dunnets post hoc analyses.

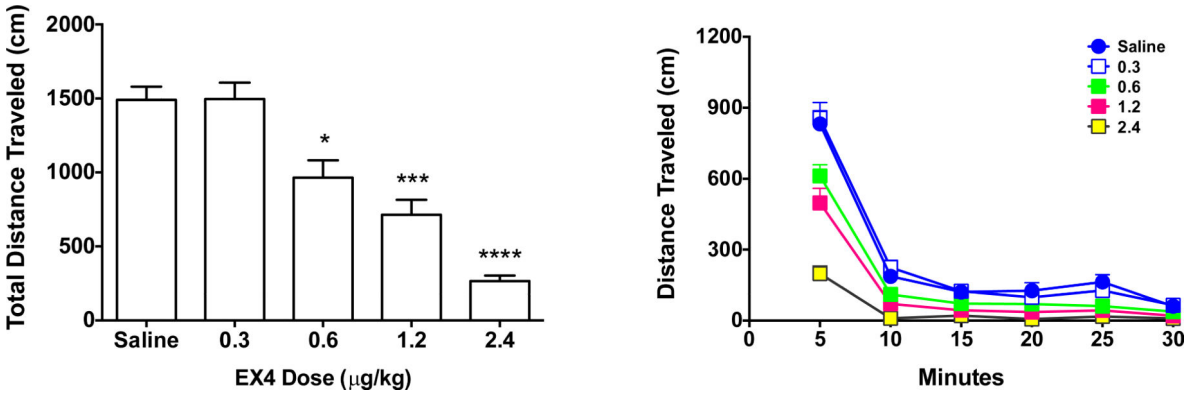

Figure 5. EX4 inhibits spontaneous locomotor activity

(A) Mean total distance traveled (± SEM) within a 30 min period. (B) Mean distance traveled (cm) during the six 5-min bins recorded. The data in panel A were analyzed with repeated measures one-way ANOVA with Dunnets post hoc analyses and significance is designated with * $p \leq 0.05$, *** $p \leq 0.001$, p **** ≤ 0.0001.

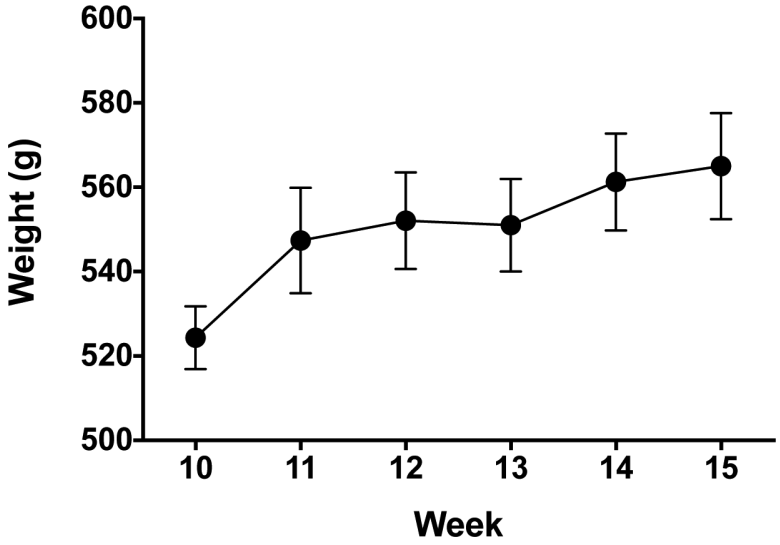

Figure 6. Normal weight gain was observed throughout the study

Circles represent the mean weights (g) (± SEM) of all subjects. Rats were weighed weekly after all experimental procedures had been completed.

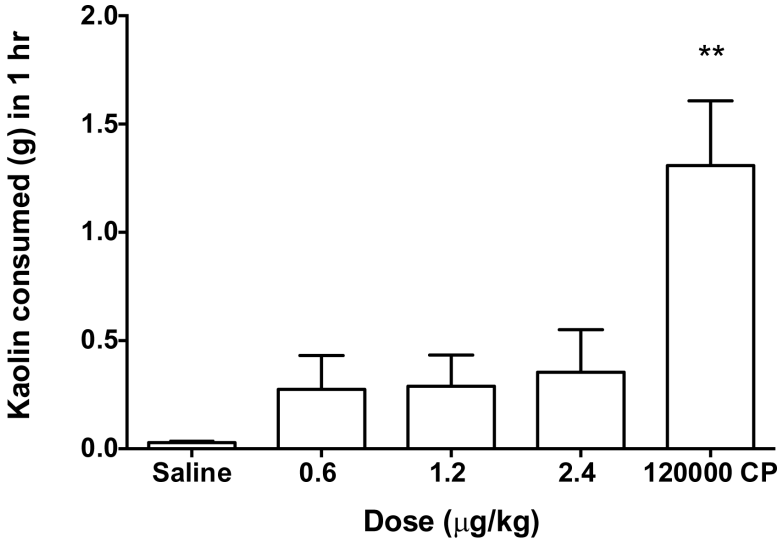

Figure 7. Acute kaolin consumption was not affected by EX4

Results depict the mean ± SEM grams of kaolin consumed in acute one hour sessions. Cyclophosphamide (CP) is shown as a positive control and is compared to saline using a students t-test, ** indicates significance with a $p \leq 0.01$.

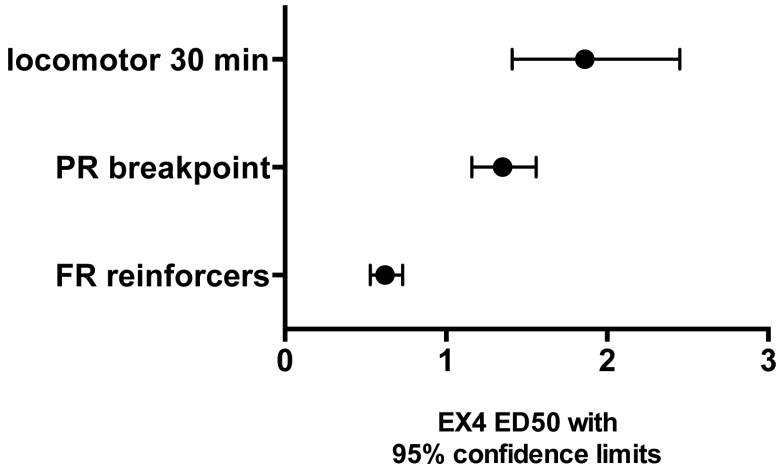

Figure 8. ED_{50} doses of EX4 with 95% confidence limits for inhibition of each behavior tested
Note that the breakpoint under PR also reflects the total number of reinforcers obtained in this schedule.