GLP-1 analog attenuates cocaine reward

Supplementary Materials and Methods

Animals and Treatment: Male wild-type C57Bl/6J mice (Jackson Laboratory, Bar Harbor, ME; 8 wks) were housed 3-5/cage and were provided with rodent chow and tap water *ad libitum*. The temperature- and humidity-controlled facility is maintained on a 12:12 h light:dark cycle (lights on 0600-1800 h). All experiments took place during the light phase of the cycle. Mice were habituated to the vivarium for a minimum of 2 wks prior to experimentation. During this time, mice were handled and ear-tagged for identification purposes. Ex-4 (10, 30, and 100 µg/kg body weight; GenScript, Piscataway, NJ), cocaine HCl (20 mg/kg; NIH/NIDA), and 0.9% saline (SAL) were administered i.p. All protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Conditioned Place Preference (CPP) Test: All testing commenced within the first 3 h of the light phase. The CPP test was divided into three phases: Preconditioning (Day 1), Conditioning (Days 2-9), and Testing (Day 10). One mouse from each cage was randomly assigned a drug treatment (pretreatment/treatment) for the CPP procedure: 10 µg/kg Ex-4/SAL, 30 µg/kg Ex-4/SAL, 100 µg/kg Ex-4/SAL, SAL/Cocaine, 10 µg/kg Ex-4/Cocaine, 30 µg/kg Ex-4/Cocaine, or 100 µg/kg Ex-4/Cocaine (N = 15-31 per group). During the Conditioning phase of the test, the treatments were alternated with SAL/SAL pairings, and treatments were counter-balanced across cohorts (i.e., half of the animals started with the drug treatment pairing; the other half, the SAL/SAL pairing). The 2-chamber CPP apparati (Med Associates, St. Albans, VT) were open field activity

chambers (28 × 28 cm) with distinct floor inserts (mesh or grid), separated by a black acrylic partition with a guillotine door, and the associated software allowed for automated analysis, as measured beam breaks on the X-Y-Z axes (16 infrared beam, 50 ms intervals). Each daily CPP session lasted 20 min. On Day 1, mice were placed into the grid-floored compartment (door removed) and were allowed to explore the chambers freely. Preference for a particular compartment (>50% of time spent in one particular chamber) was calculated and designated as the "preferred" compartment. The preferred compartment was paired with the SAL/SAL treatment condition, while the non-preferred side was paired to the drug treatment days. When a chamber preference was not evident (i.e., 50% preference for either side), the treatment pairings were assigned randomly; however, there were no cases of this during the course of the study.

On Conditioning days, mice were weighed and acclimated to the testing room for 20-30 min. The pretreatment (or SAL, on alternating days) was administered as assigned previously, and mice were returned to their home cage for 30 min. After this 30 min period, mice were given the treatment drug (or SAL on alternating days) and were placed immediately into the corresponding chamber compartment (guillotine door closed) for 20 min. Mice were returned to their home cage and were housed back in their housing room until the next day's testing.

On the Test day (day 10), mice were weighed and acclimated to the testing room as previously described and then placed into the CPP apparatus (guillotine door removed), starting on the non-preferred side. Mice were monitored for 20 min to ascertain preference for one compartment over the other.

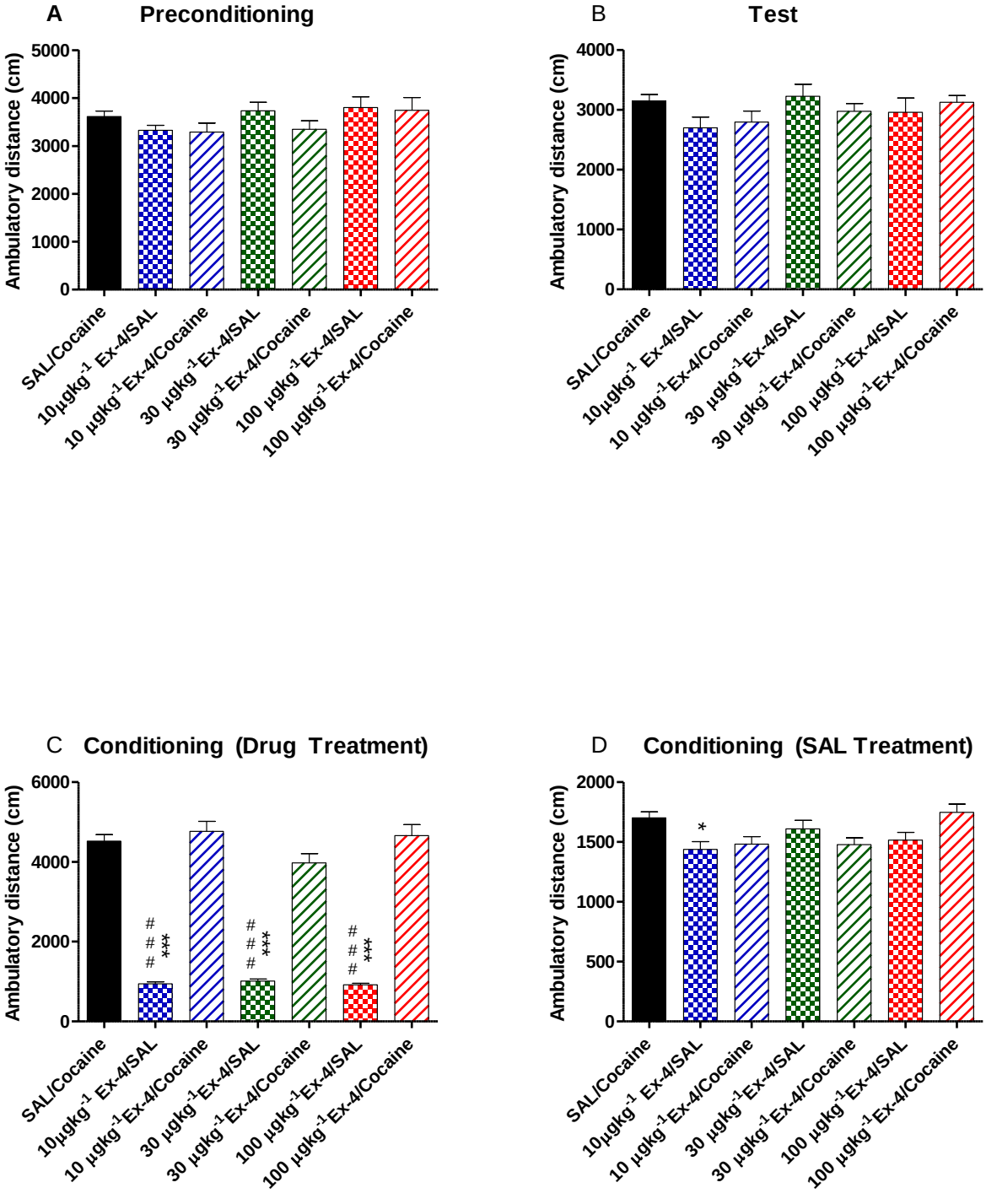
Data and Statistical Analysis: Mice were monitored for locomotor activity (e.g., ambulatory distance) and, for the Preconditioning and Test phases, time spent in each compartment. For each treatment group, calculations for the time spent in the drug treatment compartment (e.g., the non-preferred side from the preconditioning phase) on Test day relative to the time spent in that compartment on Preconditioning day were calculated (time on treatment $side_{Test}$/time on non-preferred $side_{Preconditioning}$). Values were normalized relative to that of the SAL/cocaine group for that particular cohort and expressed as a percentage relative to the SAL/cocaine group. These data were analyzed using a mixed linear model (one way ANOVA, SAS v9.3, Cary, NC) using the Proc Mixed method. Treatment was used as a factor, and data were presented as least square (LS) means ± SEM. Locomotor activity was calculated via one-way analysis of variance (ANOVA) with a *post-hoc* Bonferroni multiple comparison test using GraphPad Prism 5 (La Jolla, CA). Significance was set at $p<0.05$.

Supplementary Results and Figures

Supplemental Figure 1: Locomotor Activity during CPP. Total ambulatory distance was calculated during the Preconditioning (Suppl. Fig.1A) and Test phases (Suppl. Fig.1B), as well as during the Conditioning phase, which was collated based on the treatment given due to the balanced nature of the design. Ambulatory distance was combined for the four treatment days (when Ex-4 and/or cocaine was administered, Suppl. Fig.1C) and for the four SAL days (when only SAL was administered, Suppl. Fig.1D) as no major significant alterations were observed over time. No significant differences were apparent between the cocaine-treated groups during any of the

sessions; however, these groups had significantly elevated activity relative to the Ex-4/SAL group on treatment (but not SAL/SAL) days. Only one group (10 µg/kg Ex-4/SAL) demonstrated significantly decreased activity on the SAL treatment days relative to the SAL/cocaine group (Supplementary Figure 1D). * $p<0.05$, *** $p<0.001$ vs. SAL/Cocaine group; ### $p<0.001$ vs. respective cocaine-treated group.

Supplementary Figure 1



Supplementary Figure 1: Locomotor activity during CPP. Locomotor activity was monitored during the Preconditioning (A), Test (B), Conditioning with drug treatments (C), and Conditioning with SAL treatment (D) phases. On treatment days, cocaine administration, regardless of Ex-4 pretreatment, induced a hyperactive response in mice, which was significantly different from that found in the respective Ex-4 alone group. * $p<0.05$, *** $p<0.001$ vs. SAL/Cocaine group; ### $p<0.001$ vs. respective Ex-4/cocaine-treated group.