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Locomotor sensitization is expressed by ghrelin and D1 dopamine receptor agonist in the nucleus accumbens core in amphetamine pre-exposed rat

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ABSTRACT

Ghrelin modulates mesolimbic dopaminergic pathways in the brain in addition to its role in feeding. We investigated what roles ghrelin in the nucleus accumbens (NAcc) core may play in mediating locomotor activating effects of amphetamine (AMPH). First, when rats were administered with AMPH (1 mg/kg, i.p.) following a bilateral microinjection of ghrelin (0.1 or 0.5 μg/side) into the NAcc core, their locomotor activity was significantly enhanced, while these effects were blocked by co-microinjection of ghrelin receptor antagonist (0.5 μg/side) into this site. Second, we pre-exposed rats to saline or amphetamine (1 mg/kg, i.p.) every 2 to 3 days for a total of four times. After 2 weeks of drug-free withdrawal period, we examined the effect of saline, ghrelin (0.5 μg/side), D1 dopamine receptor agonist, SKF81297 (0.5 μg/side) or ghrelin (0.5 μg/side) + SKF81297 (0.5 μg/side) directly microinjected into the NAcc core on locomotor activity. When we measured rats' locomotor activity for 1 hour immediately following microinjections, only ghrelin + SKF81297 produces sensitized locomotor activity, while all others have no effects. These results suggest that ghrelin may have a distinct role in the NAcc core to provoke the sensitized locomotor activity induced by psychomotor stimulants, and further, it may produce these effects by interaction with D1 dopamine receptors.

Keywords amphetamine, ghrelin, locomotor activity, nucleus accumbens core, SKF81297.

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INTRODUCTION

Repeated administration of psychomotor stimulants such as amphetamine (AMPH) is well known to produce behavioral sensitization, which is expressed as enhanced increases of locomotor activity (Robinson & Berridge 1993; Vezina 2004). Once it is developed, behavioral sensitization remains for a long period of time as a form of long-term memory that contributes to the animal's drug-seeking and drug-taking behaviors (Anagnostaras, Schallert, & Robinson 2002). With these characteristics, it has been proposed as a conceptual working model for the explanation of escalating drug use and long-lasting craving in human addicts (Robinson & Berridge 1993; Vezina 2004).

Increasing evidence suggest that ghrelin, an orexigenic peptide hormone derived from the stomach (Kojima *et al.* 1999), has a regulatory role in drug addiction through interactions with mesolimbic dopaminergic pathways (Olszewski, Schiöth & Levine 2008; Ferrini *et al.* 2009; Dickson *et al.* 2011; Panagopoulos & Ralevski 2014). For example, it has shown that either systemic injection or direct microinjection into the ventral tegmental area (VTA) of ghrelin increases extracellular concentrations of dopamine (DA) in the nucleus accumbens (NAcc) and subsequent locomotor activity (Jerlhag *et al.* 2007, 2011), while direct microinjection of ghrelin into the NAcc core has no effect on basal locomotor activity (Jang *et al.* 2013). Further, it has shown that systemic injection (Wellman, Davis & Nation 2005), and direct microinjection into the NAcc core (Jang *et al.* 2013), of ghrelin enhances cocaine-induced hyper-locomotor activity in rats, while it is reduced in ghrelin-deficient knock-out mice (Abizaid *et al.* 2011) or by ghrelin receptor antagonist (Jerlhag *et al.* 2010; Jang *et al.* 2013). More recently, chronic microinjection of ghrelin

into the lateral ventricle is also shown to enhance AMPH-induced locomotor activity in rats (Sharpe *et al.* 2016). These results suggest that ghrelin may require intact dopaminergic neurotransmission originated from the VTA to regulate locomotor activity and moreover may play a certain role in producing enhanced locomotor activity similar to the locomotor sensitization as observed with repeated administrations of AMPH and cocaine (Robinson & Berridge 1993; Vezina 2004).

Evidence in the literature indicates that the NAcc is the neuronal substrate mediating the expression of behavioral sensitization (Cador, Bjijou & Stinus 1995; Zahm 2000), and the core rather than the shell is more likely involved in behavioral activation (Sellings & Clarke 2003; Li, Acerbo & Robinson 2004; Ferrario *et al.* 2005; Sellings, McQuade & Clarke 2006). However, the effects of direct microinjection of ghrelin into this site on both acute and chronic AMPH-induced locomotor activity have not been studied yet. Thus, the present study examined whether AMPH-induced locomotor activity is similarly enhanced by direct microinjection of ghrelin into the NAcc core as shown in our previous findings with cocaine (Jang *et al.* 2013) and further attempted to determine the possible role of accumbal ghrelin in the expression of locomotor sensitization after AMPH pre-exposure.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats weighing 220–250 g on arrival were obtained from Orient Bio Inc. (Seongnam-si, Korea). They were housed three per cage in a 12-hour light/dark cycle room (lights out at 8:00 pm), and all experiments were conducted during the day time. Rats had access to food and water *ad libitum* at all times. All animal use procedures were conducted according to an approved Institutional Animal Care and Use Committee protocol of Yonsei University College of Medicine. Rats were anesthetized with i.p. ketamine (100 mg/kg) and xylazine (6 mg/kg), placed in a stereotaxic instrument with the incisor bar at 5.0 mm above the interaural line and implanted with chronic bilateral guide cannulas (22 gauge; Plastics One, Roanoke, VA, USA) aimed at the NAcc core (A/P, +3.4; L, ±1.5; D/V, −7.5 mm from bregma and skull; Pellegrino, Pellegrino & Cushman 1979). Cannulas were angled at 10° to the vertical, positioned 1 mm above the final injection site and secured with dental acrylic cement anchored to stainless steel screws fixed to the skull. After surgery, 28 gauge obturators were placed in the guide cannulas, and rats were returned to their home cages for 5 to 7 days of recovery period.

Drugs and peptide

Dextroamphetamine sulfate (AMPH) (U.S. Pharmacopeia, Rockville, MD, USA) was dissolved in sterile 0.9 percent saline. Acylated rat ghrelin (American Peptide Company, Sunnyvale, CA, USA) and [D-Lys^3]-growth hormone-releasing peptide-6 (GHRP-6) (Tocris Biosciences, Ellisville, MO, USA), a ghrelin receptor antagonist, were dissolved in sterile 0.9 percent saline, and small aliquots were stored at −20°C. Immediately before use, frozen aliquots of each peptide were diluted to final working concentrations of 0.2 or 1.0 μg/l μl [equivalent to 0.06 or 0.30 nM] (ghrelin) and of 0.1 or 1.0 μg/l μl [equivalent to 0.11 or 1.08 nM] ([D-Lys^3]-GHRP-6), respectively, in 0.9 percent saline. The doses of ghrelin and its antagonist were chosen based upon our own previous report (Jang *et al.* 2013). R(+)-SKF-81297 (Sigma-Aldrich, St. Louis, MO, USA), a selective D1 DA receptor agonist, was dissolved in sterile 0.9 percent saline, and small aliquots were stored at −20°C. Immediately before use, frozen aliquots were diluted to final working concentrations of 0.2, 1.0 or 2.0 μg/l μl [equivalent to 0.54, 2.70 or 5.40 nM] in 0.9 percent saline. Bilateral intracranial microinjections into the NAcc core were made in freely moving rat. Injection cannulas (28 gauge) connected to 1-μl syringes (Hamilton, Reno, NV, USA) via PE-20 tubing were inserted to a depth 1 mm below the guide cannula tips. Injections of 0.5 μl per side were made over 30 seconds. After 1 minute, the injection cannulas were withdrawn, and the obturators were replaced.

Locomotor activity

Locomotor activity was measured with a bank of six activity boxes (35 × 25 × 40 cm; IWOO Scientific Corporation, Seoul, Korea) made of translucent Plexiglas. Each box was individually housed in a PVC plastic sound-attenuating cubicle. The floor of each box consisted of 21 stainless steel rods (5 mm diameter) spaced 1.2 cm apart center to center. Two infrared light photo beams (Med Associates, St. Albans, VT, USA) positioned 4.5 cm above the floor and spaced evenly along the longitudinal axis of the box estimated horizontal locomotor activity. Locomotor activity was counted only when two beams were consecutively interrupted. In this way, any confounding measures like grooming in a spot covering just a single beam was avoided from the counts.

Design and procedures

Once the rats had recovered from surgery, four experiments were conducted as follows: In Experiment 1, rats were randomly assigned to two groups and placed in the locomotor activity boxes for the measurement of

locomotor activity for 60 minutes while they were adjusted to the new environment. Then, each group of rats was further divided into three subgroups and bilaterally microinjected with either saline or ghrelin (0.1 or 0.5 μg/0.5 μl/side), followed by either saline or AMPH (1 mg/kg) i.p, injections for each group, respectively. They were immediately placed in the activity boxes, and their locomotor activity was measured for an additional 1 hour. Three days later, rats were bilaterally microinjected again with either saline or ghrelin (0.1 or 0.5 μg/0.5 μl/side) but this time followed by either saline or AMPH i.p. injections in a counterbalanced way; i.e. each subgroup that was formerly administered saline was now administered AMPH, and vice versa.

In Experiment 2, randomly assigned four groups of rats were allowed to stay in the locomotor activity boxes for 60 minutes to adjust to the new environment. Then, they were bilaterally microinjected first with either saline or [D-Lys^3]-GHRP-6 (0.05 or 0.5 μg/0.5 μl/side) and second with either saline or ghrelin (0.5 μg/0.5 μl/side), with an injection interval of 2 minutes. They were all followed by AMPH (1 mg/kg) i.p. injections, immediately placed in the activity boxes, and their locomotor activity was measured for 1 hour.

In Experiment 3, randomly assigned four groups of rats were allowed to stay in the locomotor activity boxes to adjust to the new environment. Then, after 60 minutes, they were bilaterally microinjected with either saline or SKF81297 (0.1, 0.5 or 1.0 μg/0.5 μl/side). They were all followed by saline i.p. injections, immediately placed in the activity boxes, and their locomotor activity was measured for 1 hour.

In Experiment 4, a total of eight groups of rats were allotted into four sets composed with two groups, respectively. Rats in each set were pre-exposed to either saline or AMPH (1 mg/kg) with a total of four i.p. injections 2–3 days apart. This regimen of drug injection is known to produce enduring sensitization of the locomotor response to amphetamine (Kim *et al.* 2001; Song *et al.* 2013). To avoid any confounding effects of conditioning, rats were administered AMPH in different places (i.e. in the activity boxes for the first and the fourth injections and in their home cages for the other injections (Kim *et al.* 2001; Song *et al.* 2013). Testing for sensitization was performed 2 weeks after the last pre-exposure injection. Rats were first habituated to the activity boxes for 60 minutes. Then, each pre-exposure group of rats was subdivided into two groups. Immediately after a bilateral microinjection with either saline, ghrelin (0.5 μg/0.5 μl/side), SKF81297 (0.5 μg/0.5 μl/side) or ghrelin + SKF81297 mixture (each as 0.5 μg/0.5 μl/side), their locomotor activity was measured for 60 minutes.

Histology

After completion of the behavioral experiments, rats were anesthetized and perfused with an intracardiac infusion of saline and 10 percent formalin. Brains were removed and further post-fixed in 10 percent formalin. Coronal sections (40 μm) were subsequently stained with cresyl violet for verification of cannula tip placements. Only rats with injection cannula tips located bilaterally in the NAcc core were included in the data analyses. For Experiment 1, out of the 35 rats surgically prepared for testing, five were dropped from the analysis for failing to meet this criterion (one in the saline, two in the low dose of ghrelin and two in the high dose of ghrelin microinjection group). For Experiment 2, out of the 32 rats surgically prepared for testing, four were dropped from the analysis for failing to meet this criterion (one in the ghrelin only and three in the ghrelin + ghrelin antagonist microinjection groups). For Experiment 3, out of the 16 rats surgically prepared for testing, no rats were dropped. For Experiment 4, out of the 75 rats surgically prepared for testing, eight were dropped from the analysis for failing to meet this criterion (two in the saline, one in the ghrelin only, two in the SKF81297 only and three in the ghrelin + SKF81297 microinjection groups). Although we verified all cannula tip placements, only those obtained from two groups are representatively depicted in Fig. c to avoid difficulty of reading due to severe overlapping.

Statistical analyses

The data were analyzed with either one-way or two-way ANOVAs, followed by Bonferroni *post hoc* comparisons. Differences between experimental conditions were considered statistically significant when $P < 0.05$.

RESULTS

Microinjection of ghrelin into the NAcc core enhances AMPH-induced hyper-locomotor activity

Following acute microinjection of ghrelin into the NAcc core, both basal and AMPH-induced locomotor activities were measured. The two-way repeated measures ANOVA with microinjections as the between factor and i.p. injections as the within factor conducted on the 1-hour total locomotor activity counts revealed multiple significant effects of microinjections [$F_{2,12} = 4.78$, $P < 0.05$] and i.p. injections [$F_{1,12} = 37.80$, $P < 0.001$] (Fig. 1a). Similar to our previous findings (Jang *et al.* 2013), AMPH-induced locomotor activity following acute microinjection of ghrelin at the dose of 0.5 μg/side into the NAcc core was significantly enhanced ($P < 0.05$ by *post hoc* Bonferroni comparison),

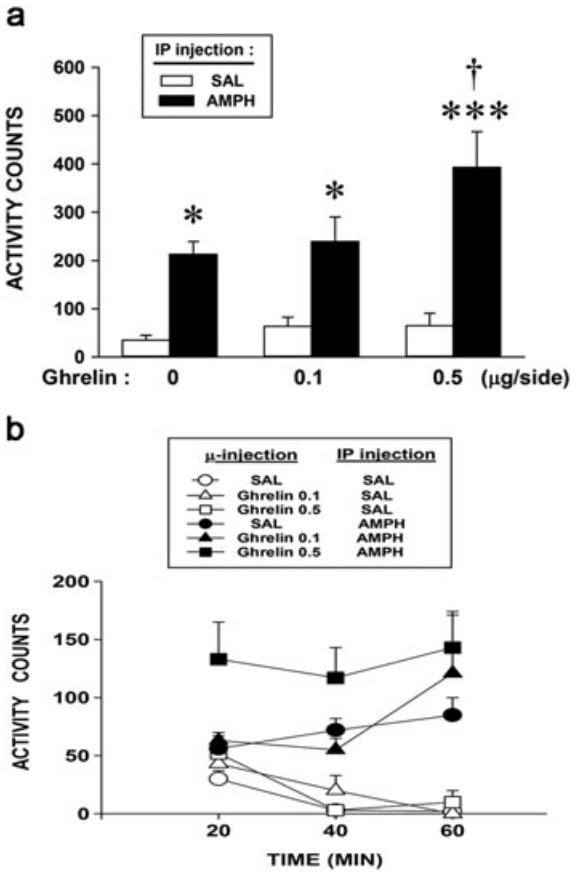

Figure 1 Microinjection of ghrelin into the NAcc core dose-dependently enhances AMPH-induced hyper-locomotor activity. Six groups of rats were bilaterally microinjected with ghrelin at the doses indicated immediately followed by either saline or AMPH (1 mg/kg, i.p.). (a) Data are shown as group mean (+S.E.M.) total locomotor activity counts observed during the 1-hour test. Symbols indicate significant differences revealed by *post hoc* Bonferroni comparisons following two-way repeated measures ANOVA. $*P < 0.05$, $***P < 0.001$, significantly more counts in AMPH relative to saline i.p. rats. $\dagger P < 0.05$, significantly more counts in ghrelin higher dose relative to saline microinjection when compared between AMPH i.p. rats ($n = 5$ per group). (b) Time-course data are shown as group mean (+S.E.M.) locomotor activity counts at 20-minute intervals obtained during the 1 hour following microinjection (saline or ghrelin) + i.p. (saline or AMPH) injection (0 to 60 minutes)

while the basal locomotor activity was not changed by ghrelin alone in the absence of AMPH. Time-course analyses showed that the ability of ghrelin in the NAcc core to enhance the AMPH-induced increase of locomotor activity was apparent throughout the 1-hour time course (Fig. 1b).

The effects of ghrelin in the NAcc core on the enhancement of AMPH-induced locomotor activity are ghrelin receptor specific

In order to determine whether the ghrelin effects on AMPH-induced locomotor activity were mediated by ghrelin receptors, a specific ghrelin receptor antagonist, [D-Lys^3]-GHRP-6, was co-microinjected with ghrelin. One-way ANOVA revealed significant differences between groups [$F_{3,24} = 6.25$, $P < 0.01$]. *Post hoc* Bonferroni comparisons revealed that the enhanced AMPH-induced locomotor activity by ghrelin in the NAcc core was inhibited by the co-presence of [D-Lys^3]-GHRP-6 (0.5 μg/side) in this site ($P < 0.05$) (Fig. 2a), suggesting

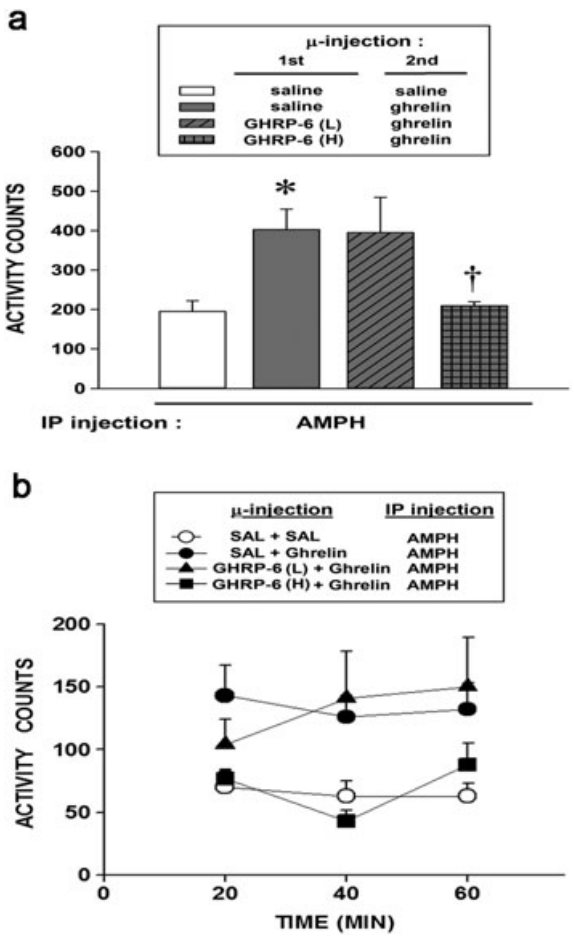

Figure 2 Co-microinjection of ghrelin receptor antagonist with ghrelin into the NAcc core inhibits the enhanced AMPH-induced hyper-locomotor activity. Four different groups of rats were all AMPH (1 mg/kg, i.p.) i.p. injected immediately following bilateral microinjections of saline, ghrelin (0.5 μg/0.5 μl/side) or ghrelin + [D-Lys^3]-GHRP-6 (0.05 or 0.5 μg/0.5 μl/side). (a) Data are shown as group mean (+S.E.M.) total locomotor activity counts observed during the 1-hour test after AMPH i.p. injections. Symbols indicate significant differences revealed by *post hoc* Bonferroni comparisons following one-way ANOVA. $*P < 0.05$, significantly more counts in ghrelin relative to saline microinjected rats. $\dagger P < 0.05$, significantly more counts in ghrelin + ghrelin antagonist relative to ghrelin alone microinjection rats ($n = 7$ for saline, 8 for ghrelin, 5 for ghrelin + ghrelin antagonist low dose, 8 for ghrelin + ghrelin antagonist high dose microinjections). (b) Time-course data are shown as group mean (+S.E.M.) locomotor activity counts at 20-minute intervals obtained during the 1 hour following microinjection (saline, ghrelin or ghrelin + ghrelin antagonist) + i.p. (AMPH) injection

that ghrelin effects were produced in its receptor specific way. The ability of [D-Lys^3]-GHRP-6 in the NAcc core to inhibit the enhanced locomotor activity observed in the rats with ghrelin alone microinjected was apparent throughout the 1-hour time course (Fig. 2b).

Previous exposure to AMPH produces sensitized locomotor activity to microinjection of ghrelin into the NAcc core when D1 DA receptor agonist co-exists

We first measured basal locomotor activity by microinjection of SKF81297 into the NAcc core. One-way ANOVA revealed significant differences between groups [$F_{3,12} = 6.37$, $P < 0.01$]. *Post hoc* Bonferroni comparisons revealed that only SKF81297 at the dose of 1.0 μg/side produced a significant basal locomotor activity compared with saline ($P < 0.05$; Fig. 3). Based upon this result, the dose of SKF81297 (0.5 μg/side), which showed no effect on basal locomotor activity by its own, was chosen for the following experiments.

Two weeks from the last pre-exposure of saline or AMPH i.p. injections, locomotor activities upon challenge microinjections of either saline, ghrelin, SKF81297 or ghrelin + SKF81297 were measured. The two-way ANOVA conducted on the locomotor activity counts revealed multiple significant effects of microinjections [$F_{3,59} = 17.01$, $P < 0.001$] and pre-exposure × microinjections interactions [$F_{3,59} = 3.33$, $P < 0.05$]. There were no differences between groups when challenged with saline, ghrelin or SKF81297 alone. Interestingly, however, AMPH compared with saline pre-exposed rats produced sensitized locomotor responses when challenged with ghrelin + SKF81297 ($P < 0.001$ by *post hoc* Bonferroni comparisons; Fig. 4a). Time-course analyses of these findings showed that the ability of ghrelin + SKF81297 to produce the sensitized locomotor activity observed in AMPH pre-exposed groups was apparent throughout the 1-hour time course (Fig. 4b). The location for the injection cannula tips used in these experiments is depicted in Fig. 4c.

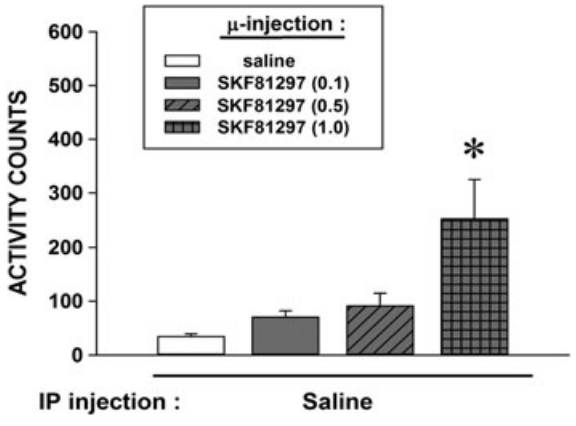

Figure 3 Basal locomotor activity was measured by microinjection of SKF81297 into the NAcc core. Four different groups of rats were all saline i.p. injected immediately following bilateral microinjections of either saline or a different dose of SKF81297 (0.1, 0.5 or 1.0 μg/0.5 μl/side). Data are shown as group mean (+S.E.M.) total locomotor activity counts observed during the 1-hour test. Symbols indicate significant differences revealed by *post hoc* Bonferroni comparisons following one-way ANOVA. *$P < 0.05$, significantly more counts in SKF81297 relative to saline microinjected rats ($n = 4$ per group)

DISCUSSION

The present results revealed that a bilateral microinjection of ghrelin into the NAcc core enhances the increase of acute AMPH-induced locomotor activity, and further, it produces the expression of locomotor sensitization in AMPH pre-exposed rats when D1 DA receptor agonist co-exists. This is the first direct demonstration, to our knowledge, that ghrelin itself in the NAcc core is able to draw the expression of AMPH-induced locomotor sensitization only with the help of D1 DA receptor-mediated neurotransmission in this site.

A full length active isoform of ghrelin receptor, growth hormone secretagogue receptor 1a (GHS-R1a), is widely distributed in various brain areas including hypothalamus, hippocampus and VTA (Guan *et al.* 1997; Zigman *et al.* 2006). Additionally, the presence of this receptor in the NAcc has also been revealed recently (Landgren *et al.* 2011; Skibicka *et al.* 2011). Based upon these findings, our present behavioral results that accumbal ghrelin enhanced AMPH-induced locomotor activity and it was blocked by its antagonist can be more naturally interpreted as these effects are mediated directly through ghrelin receptors that exist in this site. However, it is worth to mention that the ghrelin receptor antagonist, [D-Lys^3]-GHRP-6, that we used in the present study is also known to interact with 5-hydroxytryptamine (5-HT) receptors as shown in the gut (Depoortere, Thijs & Peeters 2006; Taniguchi *et al.* 2008). Although it is not known whether it is also the case in the NAcc core, the possibility that it inhibits ghrelin's locomotor enhancing effects through 5-HT receptors in our present study is very low because the concentration we used was about 100 times lower (i.e. 0.1 μM) compared with the one (i.e. 10 μM) that is known to activate 5-HT receptors resulting in effective contraction of the rat stomach fundus (Depoortere *et al.* 2006).

As generally stated in the literature, the core compared with the shell is more likely involved in the effects of drugs on behavioral activation (Zahm 2000; Sellings & Clarke 2003; Li *et al.* 2004; Ferrario *et al.* 2005; Sellings *et al.* 2006). In this regard, we targeted the core to examine the effects of ghrelin on AMPH-induced locomotor activity, and it turns out that ghrelin in this site actually enhances the increase of acute

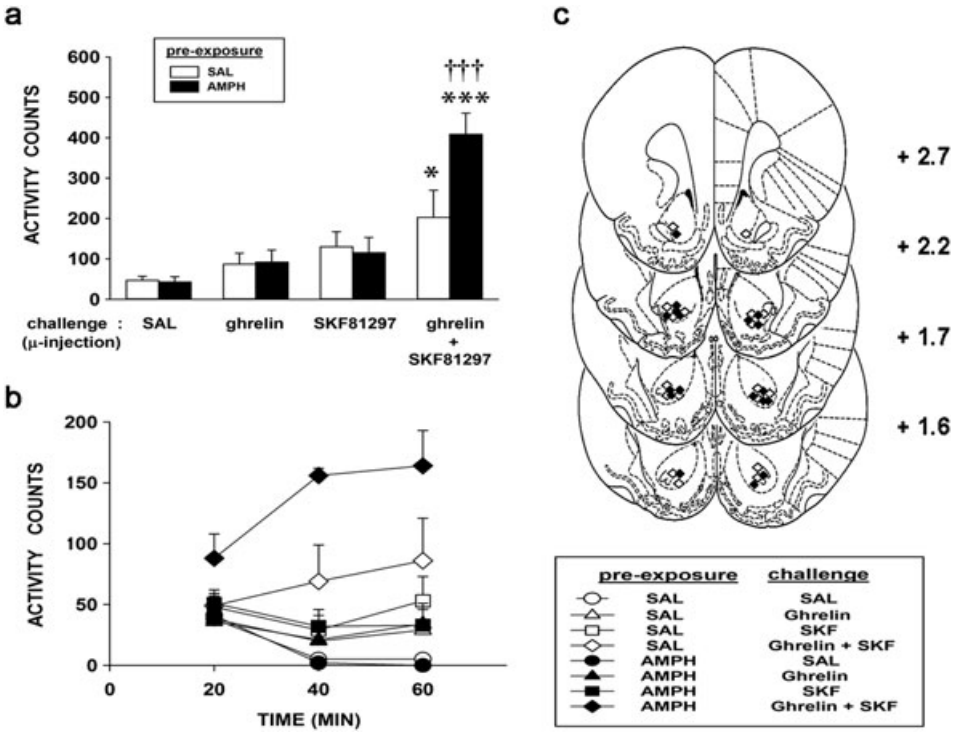

Figure 4 Previous exposure to AMPH produces sensitized locomotor activity to microinjection of ghrelin into the NAcc core when D1 DA receptor agonist co-exists. Two groups of rats were pre-exposed to either saline or AMPH (1 mg/kg, i.p.). After 2 weeks of withdrawal, each group of rats was subdivided into two groups, and the rats' locomotor activity was measured following saline, ghrelin (0.5 μg/0.5 μl/side), SKF81297 (0.5 μg/0.5 μl/side) or ghrelin + SKF81297 (0.5 μg/0.5 μl/side for each) microinjections. (a) Data are shown as group mean (+S.E.M.) total locomotor activity counts observed during the 1-hour test. Symbols indicate significant differences revealed by *post hoc* Bonferroni comparisons following two-way ANOVA. *$P < 0.05$, ***$P < 0.001$, significantly more counts relative to saline (pre-exposure)–saline (challenge) rats. †††$P < 0.001$, significant differences between AMPH pre-exposed and saline pre-exposed rats with ghrelin + SKF81297 challenge. Numbers for each group are as follows: saline (pre-exposure)–saline (challenge) (9), AMPH–saline (7), saline–ghrelin (7), AMPH–ghrelin (9), saline–SKF81297 (8), AMPH–SKF81297 (9), saline–ghrelin + SKF81297 (9) and AMPH–ghrelin + SKF81297 (9). (b) Time-course data are shown as group mean (+S.E.M.) locomotor activity counts at 20-minute intervals obtained during the 1 hour following the challenge microinjections. (c) Location of the injection cannula tips for the rats included in Fig. 4. All rats included in the data analyses had injection cannula tips located bilaterally in the NAcc core. The line drawings are from Paxinos & Watson (1997). Numbers to the right indicate millimeters from bregma. Symbols used are same as shown in (b), but only two groups (■, ◆) are shown for the purpose of visual clarity

AMPH-induced locomotor activity, similar to our previous findings with cocaine (Jang *et al.* 2013). Besides, consistent with our previous results (Jang *et al.* 2013), the present study shows again that ghrelin in the NAcc core has no effect on basal locomotor activity, indicating that ghrelin alone in this site is not enough to produce its own locomotor activity in the absence of AMPH (in the present findings) or cocaine (in the previous results). Although it has previously shown that either systemic injection or direct microinjection into the VTA of ghrelin increases locomotor activity (Jerlhag *et al.* 2007, 2011), these results may have resulted from the increased DA release in the NAcc by ghrelin action within the VTA (Jerlhag *et al.* 2007). However, it is not known yet whether ghrelin directly administered into the NAcc core influences DA-mediated neurotransmission in the same way as observed in the VTA. Related with this, it is interesting to know that cocaine- and AMPH-regulated transcript (CART) peptide, another versatile molecule involved with the regulation of both feeding and drug addiction, also has no effects on basal locomotor activity when directly administered into the NAcc core (Kim, Creekmore & Vezina 2003), whereas it increases locomotor activity when administered into the VTA (Kimmel *et al.* 2000), which are very much similar to ghrelin. These results suggest that ghrelin, or possibly even other feeding-related peptides in general, may not be sufficient to produce locomotor activity by itself when administered directly into the NAcc core but rather require other neurotransmissions (e.g. DA) activated by psychomotor stimulants directly in this site, or indirectly through the VTA, to modulate locomotor activity.

Based on our findings that ghrelin enhances acute cocaine- and AMPH-induced locomotor activities and other findings that the expression of cocaine-induced locomotor sensitization does not appear in ghrelin knock-out mice (Abizaid *et al.* 2011), we hypothesized that ghrelin in the NAcc core may have a provocative role

in drawing the expression of locomotor sensitization that normally appears by repeated injection of psychomotor stimulants (Robinson & Berridge 1993; Vezina 2004). Interestingly, we have found in the present results that just ghrelin microinjected into the NAcc core, in the absence of AMPH, actually draws the expression of locomotor sensitization only in rats previously exposed to AMPH, although these effect are observed not by ghrelin alone, but with the help of co-microinjected D1 DA receptor agonist. These results clearly indicate that ghrelin has an ability of provoking sensitized locomotor effects and further suggest that the enhanced locomotor activity observed earlier with acute cocaine or AMPH may also reflect ghrelin's such property as inducing sensitized locomotor effects. Interestingly, though, ghrelin requires some other neurotransmission co-activated in order to produce its effects, and we suggest that DA could be one of those helpers as we observed in the expression of AMPH-induced sensitization (i.e. SKF81297, a direct D1 DA receptor agonist) and in the enhancements of acute cocaine- and AMPH-induced locomotor activities (i.e. psychomotor stimulants and indirect DA receptor agonists). Overall, because systemic and direct microinjection into the VTA of ghrelin has a great chance to take DA activated mostly from the VTA, it may be enough to produce increased locomotor activity, while direct microinjection into the NAcc core of ghrelin may have no effect on DA transmission so that it requires the help from DA receptor agonists.

The fact that accumbal ghrelin requires co-activation of DA to enhance locomotor activity suggests that the neurotransmitter system activated by ghrelin itself might be not DA but rather other system, for example, may be glutamate. Interestingly, it was shown earlier in both rats and mice that ghrelin increased the activity of dopaminergic cells in the VTA, and these effects disappeared when glutamatergic neurotransmission was blocked, while they were intact when GABAergic neurotransmission was blocked (Abizaid *et al.* 2006), suggesting that glutamate system can be a target to be activated by ghrelin. Many findings in the literature indicate that glutamate plays an important role in the expression of sensitization (Wolf 1998; Vezina & Kim 1999; Kim *et al.* 2001). Interestingly, it was previously shown that either glutamate reuptake blocker or D1 DA receptor agonist, SKF82958, had no effect to bring the expression of AMPH-induced locomotor sensitization when they alone were separately microinjected into the NAcc core, whereas the expression of sensitization was successfully produced when they were simultaneously co-microinjected into the same site (Kim *et al.* 2001). Very similar to these findings, our present results also show that either ghreline or D1 DA receptor agonist, SKF81297, fails to induce the expression of AMPH-induced locomotor sensitization when only one of them were separately microinjected into the NAcc core, whereas it was successfully produced when both were co-microinjected into this site, implying that ghrelin may do play a role in increasing glutamate neurotransmission as glutamate reuptake blocker does. Although this is very much a speculative idea, the recent findings in the hippocampus that ghrelin triggers the synaptic incorporation of glutamate receptors (Ribeiro *et al.* 2014) help support the notion that ghrelin may enhance glutamatergic neurotransmission in the NAcc core as well. However, it completely remains in the future to be explored whether ghrelin actually recruits glutamate system in the context of AMPH-induced locomotor activity.

In conclusion, the present findings indicate that ghrelin in the NAcc core enhances acute AMPH-induced hyper-locomotor activity. Further, ghrelin in this site produces sensitized locomotor activity in the presence of D1 DA receptor agonist in AMPH pre-exposed rats. These results expand the role of accumbal ghrelin to the expression of locomotor sensitization and further suggest the possible interaction of DA and other neurotransmitter system (e.g. glutamate) involved in these processes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

JKJ, WYK and J-HK designed the experimental strategy and analyzed the data. JKJ, WYK, BRC and JWL performed all the experiments. J-HK wrote the manuscript. All authors critically reviewed content and approved final version for publication.

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