Neurochemistry International 124 (2019) 25–30

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

A novel synthetic cathinone, α-pyrrolidinopentiothiophenone (PVT), produces locomotor sensitization in rat: Implications for GSK3β connections in the nucleus accumbens core

Hyung Shin Yoon, Min Jeong Ku, Wen Ting Cai, Jeong-Hoon Kim*

Department of Physiology, Brain Korea 21 Plus Project for Medical Science, Brain Research Institute, Yonsei University College of Medicine, Seoul, 03722, South Korea

ARTICLE INFO

Keywords:
α-PVT
Novel psychoactive substance
Locomotor sensitization
Nucleus accumbens
GSK3β

ABSTRACT

A novel psychoactive substance, α-pyrrolidinopentiothiophenone (α-PVT), is a structural analog to amphetamine. Recently, it has been shown that α-PVT has an abuse potential similar to psychomotor stimulants like cocaine or amphetamine. However, it has not been performed yet to determine whether α-PVT develops behavioral sensitization, a well-known phenomenon for psychomotor stimulants. In the present study, rats were first pre-exposed to either saline or α-PVT (20 mg/kg, IP) with a total of four injections in every 2–3 days of interval. Then, 2-weeks after withdrawal, locomotor activity was measured with a challenge dose (10 mg/kg, IP) of α-PVT and the nucleus accumbens core region was taken out. Similar to psychomotor stimulants, repeated administration of α-PVT produced locomotor sensitization. Further, the phosphorylation levels of GSK3β in the nucleus accumbens core were found to be decreased only in rats with sensitization developed, but not in those with acute or non-sensitized. Correlation analysis revealed that the phosphorylation levels of GSK3β have a strong negative correlation with locomotor activity only in rats with α-PVT pre-exposed, but not in those with its acute injection. These results suggest that a certain level of change in the phosphorylation levels of GSK3β in the nucleus accumbens core may involve in mediating the expression of locomotor sensitization by repeated injection of α-PVT in rats.

1. Introduction

The increased use of novel psychoactive substances as recreational drugs has become substantial health risk around the world in recent years (Welter-Luedeke and Maurer, 2016). One such substance with stimulant properties, α-pyrrolidinopentiothiophenone (α-PVT), first appeared on several online drug forums in 2012 (Swortwood et al., 2016) and was identified in Japan in 2013 (Uchiyama et al., 2014). It is a novel cathinone derivative with a thiophene ring replacing the phenyl ring and is known to inhibit monoamine transporters with significant neurotoxicity (Eshleman et al., 2017; Marusich et al., 2014; Wojcieszak et al., 2016), suggesting that it has a similar property with psychomotor stimulants. Supporting this, it has been recently demonstrated that α-PVT has an abuse potential with rewarding and discriminative stimulus effects similar to cocaine or methamphetamine (Cheong et al., 2017; Gatch et al., 2017).

It is well known that psychomotor stimulants such as amphetamine, when repeatedly administered to rodents, further enhances the increased locomotor activity by acute drug injection, called locomotor sensitization (Vezina, 2004). As sensitization, once developed, remains as a form of long-term memory, it is thought to contribute to the animal's drug-seeking and -taking behaviors (Anagnostaras et al., 2002). Further, it has been proposed as a conceptual working model which explains certain characteristics of human drug addicts such as escalating drug use and long-lasting craving (Robinson and Berridge, 1993; Vezina, 2004). However, in spite of α-PVT's abuse potential, it has not been reported yet what role α-PVT plays in the development of locomotor sensitization.

The nucleus accumbens (NAcc), consisting of core and shell as two sub-regions, is an important neuronal substrate mediating the locomotor activating and rewarding effects of drugs of abuse (Goto and Grace, 2008; Hyman, 1996; Koob and Le Moal, 2001; Robbins et al., 1989; Vezina, 2004). Interestingly, it is known that phosphorylation levels for glycogen synthase kinase 3 β (GSK3β), a serine/threonine kinase abundantly present in the brain, in the NAcc core, but not in shell, were decreased following acute cocaine administration (Kim et al., 2013; Perrine et al., 2008). In parallel with this, there are many reports showing that GSK3β contributes to psychomotor stimulant-

* Corresponding author. Department of Physiology, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul, 03722, South Korea.
E-mail address: jkim1@yuhs.ac (J.-H. Kim).

https://doi.org/10.1016/j.neuint.2018.12.005
Received 17 October 2018; Received in revised form 15 November 2018; Accepted 13 December 2018
Available online 18 December 2018
0197-0186/

induced characteristic behaviors (Beaulieu et al., 2007, 2009). For example, inhibition of GSK3β activity by systemic injection of a specific inhibitor, SB216763, attenuates acute cocaine- or amphetamine-induced hyper-activity (Enman and Unterwald, 2012; Miller et al., 2009), while microinjection of SB216763 into the NAcc core, but not in the shell, blocks the expression of locomotor sensitization produced by repeated injection of cocaine or methamphetamine (Xu et al., 2009, 2011). These results suggest that a certain level of intact GSK3β activity in the NAcc core is necessary for psychomotor stimulant-induced locomotor activity.

Thus, in the present study, we examined whether locomotor sensitization is similarly developed by α-PVT as shown in our previous findings with amphetamine (Kim et al., 2001; Song et al., 2013), and further examined to find the possible role of GSK3β in the NAcc core in such effects.

2. Materials and methods

2.1. Subjects and drugs

Male Sprague-Dawley rats weighing 220–250 g (equivalent to 6 weeks olds) on arrival were obtained from Orient Bio Inc. (Seongnam-si, Korea). They were housed three per cage in a 12-hr light/dark cycle room (lights out at 8:00 p.m.), and all experiments were conducted during the day time. The 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.

α-PVT was synthesized and kindly provided by Professor Yong Sup Lee at the Department of Pharmacy, Kyung Hee University (Seoul, South Korea). It was dissolved to a final working concentration of 20 mg/ml in 0.9% saline. The dose of α-PVT was chosen based upon previous report (Gatch et al., 2017).

2.2. 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 photobeams (Med Associates, St. Albans, VT, USA), positioned at 4.5 cm above the floor and spaced evenly along the longitudinal axis of the box, were used to estimate horizontal locomotor activity.

2.3. Design and procedures

A schematic illustration showing time lines for the entire experimental design was depicted in Fig. 1.

Upon arrival, all rats passed a week-long adaptation period to the new housing environment, and experiments were conducted as follows. Two groups of rats (equivalent to approximately 7 weeks olds) were pre-exposed to saline or α-PVT (20 mg/kg) with a total of four intraperitoneal (IP) injections 2–3 days apart. This regimen of drug injection is known to produce enduring sensitization of the locomotor response to psychomotor stimulants like amphetamine (Kim et al., 2001; Song et al., 2013). To avoid any confounding effects of conditioning, rats were administered α-PVT 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). Only during the first and the fourth injections, rats were first habituated to the activity boxes for 30 min, and their locomotor activity was measured for additional 60 min immediately following saline or α-PVT injections. Testing for sensitization was performed 2 weeks after the last pre-exposure injection. Rats (equivalent to approximately 10 weeks olds by this time) were first habituated to the activity boxes for 60 min. Then, each pre-exposure group of rats was sub-divided into two groups. Immediately after systemic injection with either saline or α-PVT (10 mg/kg, IP), their locomotor activity was measured for 60 min. Upon α-PVT challenge, if locomotor activity in α-PVT pre-exposed group is equal to or lesser than the mean value of locomotor activity in saline pre-exposed group, then it is considered as non-sensitized. Otherwise, it is categorized as sensitized. A total of 41 rats was used and included in the statistical analysis in this study.

2.4. Brain tissue preparation

Animals were decapitated by guillotine after 60 min of locomotor activity measurement following saline or α-PVT IP challenge injections. Brains were rapidly removed and coronal sections (1.0 mm thick extending 1.60–2.60 mm from bregma) were obtained with an ice-cold brain slicer. Tissue punches (1.2 mm diameter) were obtained in the NAcc core region on an ice-cold plate, immediately frozen on dry ice and stored at −80 °C. They were prepared bilaterally and pooled for each individual animal's protein isolation.

2.5. Western blotting

Tissues were homogenized in lysis buffer containing 0.32 M sucrose, 2 mM EDTA, 1% SDS, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium fluoride, and 1 mM sodium orthovanadate. The concentration of protein was determined by using Pierce Coomasie Protein Assay Kit (Thermo Scientific Inc., Rockford, IL). Samples were then boiled for 10 min and subjected to SDS-polyacrylamide gel electrophoresis. Proteins (10 μg and 12 μg per lane for total and phosphorylated GSK3β, respectively) were separated and transferred electrophoretically to nitrocellulose membranes (Bio-Rad, Hercules, CA), which were then blocked with 5% bovine serum albumin (BSA) in PBS-T buffer [10 mM phosphate-buffered saline plus 0.05% Tween-20]. Antibodies used to probe the blots were as following:

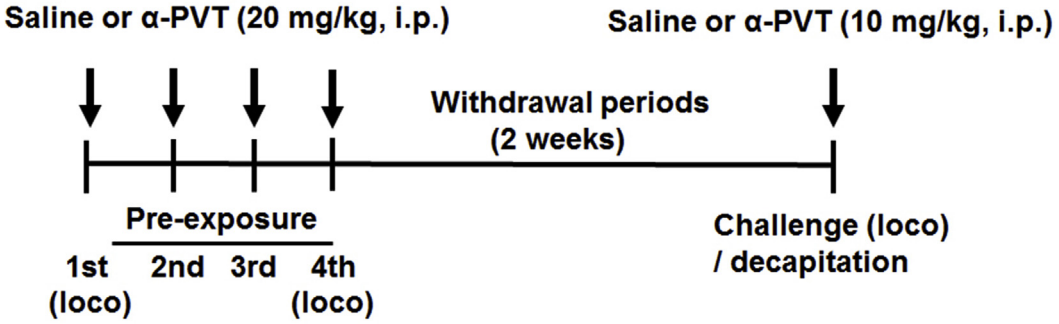

Fig. 1. Time lines for the whole experimental procedures were illustrated with the points where saline or α-PVT injected. Note that locomotor activity was not measured during the 2nd and 3rd pre-exposures.

Table 1
Locomotor activity counts during pre-exposures.

Pre-exposure	Day 1	Day 4
saline (20)	35 ± 7	45 ± 8
α-PVT (21)	381 ± 71***	392 ± 57***

All rats were habituated for 30 min and their locomotor activity measured for an additional 60 min following their respective injections. Only at day 1 and 4, locomotor activity was measured during the pre-exposure injections (once daily total 4 injections with 2–3 days interval). *** $p < 0.001$, significant differences in α-PVT compared to saline pre-exposed animals as revealed by *post hoc* Tukey comparisons following two-way repeated measure ANOVA. Numbers in parentheses indicate n/group.

total GSK3β (1:4000), phospho-Ser9-GSK3β (1:2000), purchased from Cell Signaling (Beverly, MA) and diluted in PBS-T with 5% BSA; β-actin (1:10,000), purchased from Abcam (Cambridge, UK) and diluted in PBS-T with 5% skim milk. Two separate gels were used to detect total and phosphorylated proteins, respectively. Primary antibodies were detected with peroxidase-conjugated secondary antibodies, anti-rabbit IgG (1:2000; KOMA Biotech, Seoul, Korea) diluted in PBS-T with 5% skim milk, followed by enhanced chemiluminescence (ECL) reagents (Amersham Biosciences, Arlington Heights, IL) and exposure to X-ray film. Band intensities were quantified based on densitometric values using Fujifilm Science Lab 97 Image Gauge software (version 2.54) (Fujifilm, Tokyo, Japan).

2.6. Statistical analyses

Statistical analyses were performed using the Sigma Plot version 12.0 (Systat Software, San Jose, CA). The data in Table 1 and Fig. 2B were analyzed using two-way repeated-measure ANOVA (analysis of variance). The data in Fig. 2A and 3 were separately analyzed by two-way ANOVA followed by Tukey post-hoc comparisons [for the former four groups with a different combination of pre-exposure and challenge (sensitized)], and by additional *t*-test [for the last two challenged groups, sensitized and non-sensitized]. Correlation analyses were assessed by Spearman's rank correlation coefficients (*r*). Differences between experimental conditions were considered statistically significant when $P < 0.05$.

3. Results

3.1. α-PVT produces locomotor sensitization

We first measured locomotor activity during pre-exposure of α-PVT (20 mg/kg, IP). The two-way repeated measures ANOVA conducted on the 60 min total locomotor activity counts, on both day 1 and day 4, revealed a significant effect of α-PVT compared to saline [$F_{1,39} = 41.99$, $p < 0.001$], while there was no significant difference detected between days (Table 1). Interestingly, however, after 2-weeks of drug-free withdrawal period, when we measured locomotor activity upon α-PVT (10 mg/kg, IP) challenge injection, α-PVT compared to saline pre-exposed group showed a significantly increased locomotor activity ($p < 0.01$). The two-way ANOVA conducted on the 60 min total locomotor activity counts revealed multiple significant effects of pre-exposure [$F_{1,32} = 6.59$, $p = 0.015$], challenge [$F_{1,32} = 60.18$, $p < 0.001$], and pre-exposure × challenge interactions [$F_{1,32} = 7.55$, $p = 0.01$] (Fig. 2A). Our regimen produced well-developed sensitization responses in terms of locomotor activity for α-PVT (20 mg/kg), although out of 13 rats, α-PVT pre-exposed and α-PVT challenged, we found that only 8 was sensitized (about 62%), which is still within a common range compared to psychomotor stimulants like amphetamine (normally 60–80% depending on batch). The *t*-test conducted on the 60 min total locomotor activity counts between sensitized and non-sensitized groups revealed a significant difference [$t_{1,11} = 2.80$, $p = 0.017$]. Locomotor activity counts during time-courses for pre- and post-challenge injection in different groups are shown in Fig. 2B. No significant differences between groups were detected during habituation (pre-injection of challenge), while the sensitizing effect of α-PVT on locomotor activity was mostly appeared during the first 20 min after challenge injection ($p < 0.001$).

3.2. Expression of α-PVT-induced locomotor sensitization has a significant correlation between locomotor activity and the phosphorylation levels of GSK3β in the NAcc core

In order to examine whether α-PVT may regulate the phosphorylation levels of GSK3β in the NAcc core, we measured the ratio of the phosphorylated to total GSK3β levels by western blot with the NAcc core tissues obtained (Fig. 3A) at 60 min after saline or α-PVT challenge injection. The two-way ANOVA conducted on these data showed multiple significant effects of pre-exposure [$F_{1,32} = 20.54$, $p < 0.001$] and

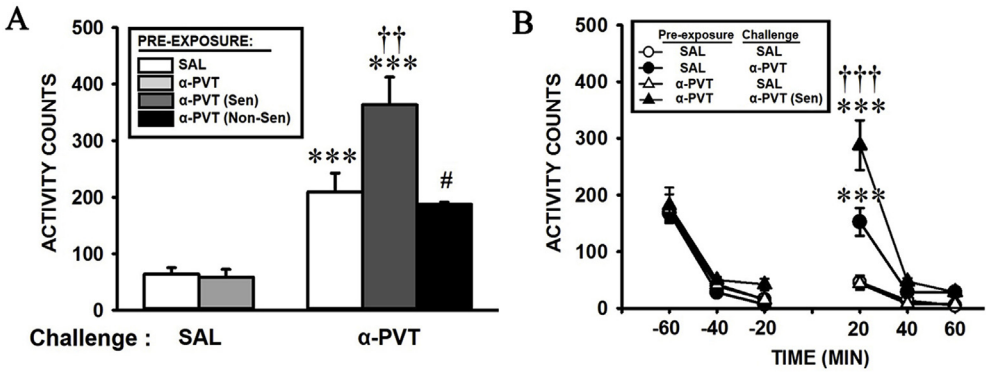

Fig. 2. Repeated systemic administration of α-PVT produces locomotor sensitization. Two groups of rats were pre-exposed to either saline or α-PVT (20 mg/kg, IP). After 2 weeks of withdrawal, each group of rats was sub-divided into two groups. All rats were habituated for 60 min and their locomotor activity measured for an additional 60 min following either saline or α-PVT (10 mg/kg, IP) injection. (A) Data are shown as group mean (+S.E.M.) total locomotor activity counts observed during the 60 min test. Numbers for each group are as follows: saline (pre-exposure) – saline (challenge) (11), α-PVT – saline (8), saline – α-PVT (9), α-PVT (sensitized) – α-PVT (8), and α-PVT (non-sensitized) – α-PVT (5). Symbols indicate significant differences revealed by post-hoc Tukey comparisons following two-way ANOVA for the former 4 groups, or by *t*-test for the last two groups. For the purpose of easy comparisons, both sensitized and non-sensitized sub-groups were shown side by side with other groups in a single graph. *** $p < 0.001$, significantly more counts in α-PVT - relative to saline-challenged rats with saline and α-PVT pre-exposure, respectively. †† $p < 0.01$, significant differences between α-PVT pre-exposed and saline pre-exposed rats with α-PVT challenge. # $p < 0.05$, significant differences between sensitized and non-sensitized rats in α-PVT pre-exposed and α-PVT challenged group. (B) Time-course data are shown as group mean (± S.E.M.) locomotor activity counts at 20 min intervals obtained during the 1 hour preceding (−60 through 0 min) and the 1 hour following the challenge (saline or α-PVT) injection (0–60 min). Symbols indicate significant differences revealed by post-hoc Tukey comparisons following two-way ANOVA. *** $p < 0.001$, significantly more counts in α-PVT - relative to saline-challenged rats with saline and α-PVT pre-exposure, respectively. ††† $p < 0.001$, significant differences between α-PVT pre-exposed and saline pre-exposed rats with α-PVT challenge.

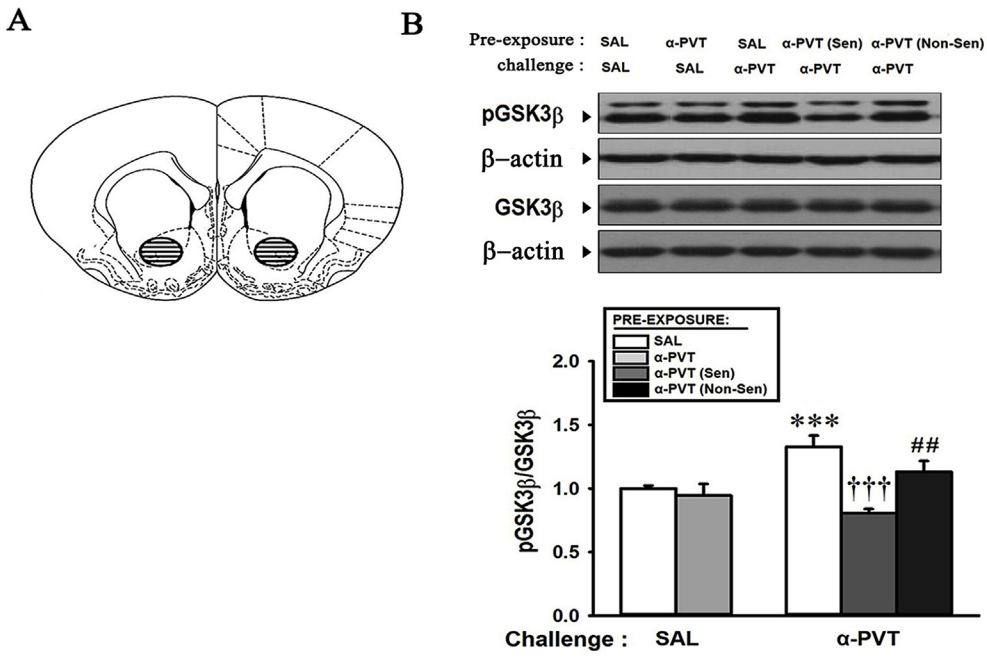

Fig. 3. Phosphorylation levels for GSK3β in the NAcc core were changed depending on α-PVT state. The NAcc core tissues were punched out 60 min after either saline or α-PVT (10 mg/kg) challenge IP injection 2 weeks after pre-exposure of saline or α-PVT. (A) The NAcc core region where tissues were taken out is shown (cross-hatched circles). Punches (1.2 mm diameter) were prepared bilaterally and pooled for each individual animal's protein isolation. Line drawing is from Paxinos and Watson (2004) and depicts the caudal surface of a coronal section (1.0 mm thick) extending 1.70–2.70 mm from bregma. (B) Representative Western blots were shown. Values for the band intensities were first normalized to β-actin and then the average values for the ratio of phosphorylated to total proteins in each group were expressed as mean + s.e.m. relative to saline pre-exposure + saline challenge control group. Numbers for each group are the same as shown in Fig. 2. Symbols indicate significant differences as revealed by *post-hoc* Tukey comparisons following two-way ANOVA for the former 4 groups, or by *t*-test for the last two groups. For the purpose of easy comparisons, both sensitized and non-sensitized sub-groups were shown side by side with other groups in a single graph. ***$p < 0.001$, significantly different from saline pre-exposed rats with saline challenge. ††† $p < 0.001$, significant differences between saline pre-exposed and α-PVT pre-exposed rats with α-PVT challenge. ## $p < 0.01$, significant differences between sensitized and non-sensitized rats in α-PVT pre-exposed and α-PVT challenged group.

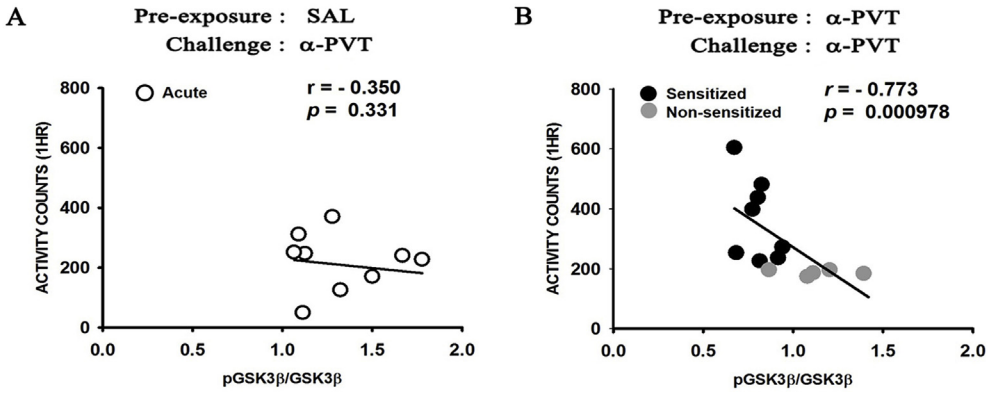

Fig. 4. Correlation analysis between locomotor activity and phosphorylation levels of GSK3β. Correlation was analyzed using the Spearman rank-order on data obtained from α-PVT challenged rats. (A) There was no significant correlation between locomotor activity and phosphorylation levels of GSK3β in saline pre-exposed with α-PVT challenged (i.e., acute α-PVT) rats. (B) A significant negative correlation was observed between locomotor activity and phosphorylation levels of GSK3β in α-PVT pre-exposed with α-PVT challenged rats.

pre-exposure × challenge interactions [$F_{2,35} = 13.43$, $p < 0.001$]. Post hoc Tukey comparisons revealed that α-PVT compared to saline challenge injection produced significantly higher ratio of phosphorylated to total GSK3β in saline pre-exposed rats ($p < 0.001$), while these effects were absent in α-PVT pre-exposed rats (Fig. 3B). Interestingly, however, these effects were appeared only in rats, showing locomotor sensitization. The *t*-test conducted on the ratio of phosphorylated to total GSK3β between sensitized and non-sensitized groups revealed a significant difference [$t_{1,11} = 2.80$, $p = 0.002$]. Correlation analysis for the data obtained from the rats with α-PVT pre-exposure that were α-PVT challenged indicated that there were significant negative correlation between locomotor activity and the ratio of phosphorylated to total GSK3β (r = - 0.77, $p < 0.001$) (Fig. 4B). There were no significant correlation for the data obtained from the rats with saline pre-exposure that were α-PVT challenged (r = - 0.35, $p = 0.331$) (Fig. 4A).

4. Discussion

The present results demonstrated that repeated systemic injection of α-PVT produces locomotor sensitization similar to amphetamine. In addition, we showed that the expression of this effect was negatively correlated with decrease of phosphorylation levels of GSK3β in the NAcc core. This is the first demonstration, to our knowledge, to indicate that α-PVT has an ability producing behavioral sensitization and that GSK3β is involved in mediating this behavior.

Recently, it has been demonstrated that acute systemic injection of α-PVT produced a significant stimulatory locomotor activity in mice at doses between 10 and 50 mg/kg (Gatch et al., 2017). Based upon these findings, α-PVT was repeatedly administered in rat at a dose of 20 mg/kg during pre-exposure and 10 mg/kg for a challenge in our present experiments. As shown in Fig. 2, our experimental scheme fairly well produced locomotor sensitization at this dose. Considering that amphetamine produces locomotor sensitization with a dose of as low as 1 mg/kg, the requiring dose that we need to produce locomotor sensitization is relatively higher for α-PVT compared to amphetamine, and it may reflect their different potency to monoamine transporters (Eshleman et al., 2017; Marusich et al., 2014; Wojcieszak et al., 2016). Because the occurrence of the expression of behavioral sensitization implies that the incentive salience attribution is sensitized to representations of drug-related cues as affluently shown in the literature

(Robinson and Berridge, 1993, 2003; Vezina, 2004), the present results that α-PVT produces locomotor sensitization suggest that, when repeatedly administered, it has an abuse potential similar to psychomotor stimulants. Supporting this, it has been recently shown that α-PVT produces a significant conditioned place preference in mice and increases its self-administration in rats (Cheong et al., 2017). Taken together, these results clearly indicate that α-PVT has a certain level of abuse potential in diverse aspects similar to other well-known psychomotor stimulants like amphetamine or cocaine.

There are quite amount of studies showing that phosphorylation levels for GSK3β at serine 9 residue in the NAcc, especially in the core, but not in the shell, contribute to the expression of psychomotor stimulants-induced locomotor sensitization. For example, chronic administration of cocaine reduces the phosphorylation levels of GSK3β in the NAcc core (Kim et al., 2013), while the increase of phosphorylation levels for GSK3β in this site by lithium chloride or valproic acid attenuates the psychomotor stimulants-induced locomotor sensitization (Enman and Unterwald, 2012; Xu et al., 2009, 2011). Similar to these results, the phosphorylation levels for GSK3β at serine 9 residue in the NAcc core, in our present findings, were reduced by chronic α-PVT compared to those expressed by acute α-PVT; interestingly, however, these effects were appeared only in rats with locomotor sensitization expressed, but not in those with non-sensitized (Fig. 3B). These results suggest that the change of phosphorylation levels for GSK3β in the NAcc core may importantly contribute to the expression of α-PVT-induced locomotor sensitization similar to what already observed with other psychomotor stimulants. Supporting this possibility, we observed that there is a good negative correlation between locomotor activity and the phosphorylation levels for GSK3β, only in chronic α-PVT groups, but not in acute α-PVT group (Fig. 4). In the literature, it has been shown that, aside from well-known canonical dopamine (DA) signaling pathway, D2-like DA receptors are also able to exert their effects through an Akt-GSK3β signaling cascade in a cAMP-independent manner (Beaulieu et al., 2007, 2009). So, in consideration of our present findings that there is a GSK3β correlation, it is possible that repeated administration of α-PVT may produce locomotor sensitization by a similar mechanism involving D2 dopamine receptor signaling pathways. From GSK3β to actual locomotor activity, it is hard to delineate which one, among the multiple downstream effectors, GSK3β may target to produce such effects. Interestingly, there have been some reports that GSK3β may regulate AMPA receptor trafficking and function (Lee et al., 2018; Peineau et al., 2007; Wei et al., 2010), suggesting that it is possible for GSK3β to produce its effect on locomotor sensitization through this signaling pathway. It all remains in the future to find out how they might work.

With regard to the phosphorylation levels for GSK3β in the NAcc core, it is worth to mention that α-PVT, when acutely administered, rather increased its levels differently from acute cocaine or amphetamine that decreased its levels, although they all produced the increase of locomotor activity (Kim et al., 2013; Miller et al., 2009; Xu et al., 2009, 2011). These results may suggest that signaling pathways activated by α-PVT in terms of producing locomotor activity might be different from those by cocaine or amphetamine. However, careful examination of the phosphorylation levels for GSK3β in the NAcc core and locomotor activity in the previous literature suggest that the mere high or low phosphorylation levels for GSK3β in the NAcc core do not directly indicate that rats will show decreased or increased locomotor activity, accordingly. For example, microinjection of S9 peptide, GSK3β activator, into the NAcc core decreases phosphorylation levels for GSK3β, but there is no locomotor activity changed by itself, and rather it requires cocaine stimulation (Kim et al., 2013). Further, it has been shown that, very similar to our findings, cocaine challenge in saline pre-exposed rats produces the increase of phosphorylation levels for GSK3β in the NAcc core, but this increase drops down back to control level by cocaine challenge in cocaine pre-exposed rats accompanied with locomotor sensitization (Nwaneshiudu and Unterwald, 2010). Taken together, these results suggest that a relative change of the phosphorylation levels for GSK3β in the NAcc core contributes to the locomotor activity, especially to the expression of locomotor sensitization, in a context dependent manner.

In conclusion, our present results indicate that α-PVT produces locomotor sensitization in a similar way to other psychomotor stimulants, and further suggest that its effects on the expression of locomotor sensitization are negatively correlated with the phosphorylation levels for GSK3β in the NAcc core. It remains to be determined in the future how α-PVT produces its effects on locomotor sensitization, especially through GSK3β mediated signaling pathways.

Acknowledgements

This research was supported by a grant (15182MFDS485) from the Ministry of Food and Drug Safety, Republic of Korea, in 2015 (J.-H.K.)

References

Anagnostaras, S.G., Schallert, T., Robinson, T.E., 2002. Memory processes governing amphetamine-induced psychomotor sensitization. Neuropsychopharmacology 26, 703–715.
Beaulieu, J.M., Gainetdinov, R.R., Caron, M.G., 2007. The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol. Sci. 28, 166–172.
Beaulieu, J.M., Gainetdinov, R.R., Caron, M.G., 2009. Akt/GSK3β signaling in the action of psychotropic drugs. Annu. Rev. Pharmacol. Toxicol. 49, 327–347.
Cheong, J.H., Choi, M.J., Jang, C.G., Lee, Y.S., Lee, S., Kim, H.J., Seo, J.W., Yoon, S.S., 2017. Behavioral evidence for the abuse potential of the novel synthetic cathinone alpha-pyrrolidinopentiothiophenone (PVT) in rodents. Psychopharmacology (Berlin) 234, 857–867.
Enman, N.M., Unterwald, E.M., 2012. Inhibition of GSK3β attenuates amphetamine-induced hyperactivity and sensitization in the mouse. Behav. Brain Res. 231, 217–225.
Eshleman, A.J., Wolfrum, K.M., Reed, J.F., Kim, S.O., Swanson, T., Johnson, R.A., Janowsky, A., 2017. Structure-activity relationships of substituted cathinones, with transporter binding, uptake, and release. J. Pharmacol. Exp. Therapeut. 360, 33–47.
Gatch, M.B., Dolan, S.B., Forster, M.J., 2017. Locomotor activity and discriminative stimulus effects of a novel series of synthetic cathinone analogs in mice and rats. Psychopharmacology (Berlin) 234, 1237–1245.
Goto, Y., Grace, A.A., 2008. Limbic and cortical information processing in the nucleus accumbens. Trends Neurosci. 31, 552–558.
Hyman, S.E., 1996. Addiction to cocaine and amphetamine. Neuron 16, 901–904.
Kim, J.H., Perugini, M., Austin, J.D., Vezina, P., 2001. Previous exposure to amphetamine enhances the subsequent locomotor response to a D1 dopamine receptor agonist when glutamate reuptake is inhibited. J. Neurosci. 21, RC133.
Kim, W.Y., Jang, J.K., Lee, J.W., Jang, H., Kim, J.H., 2013. Decrease of GSK3β phosphorylation in the rat nucleus accumbens core enhances cocaine-induced hyper-locomotor activity. J. Neurochem. 125, 642–648.
Koob, G.F., Le Moal, M., 2001. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24, 97–129.
Lee, J.W., Kim, W.Y., Kim, J.H., 2018. Leptin in the nucleus accumbens blocks the increase of GluA1 phosphorylation induced by acute cocaine administration. Neuroreport 29, 483–487.
Marusich, J.A., Antonazzo, K.R., Wiley, J.L., Blough, B.E., Partilla, J.S., Baumann, M.H., 2014. Pharmacology of novel synthetic stimulants structurally related to the "bath salts" constituent 3,4-methylenedioxypyrovalerone (MDPV). Neuropharmacology 87, 206–213.
Miller, J.S., Tallarida, R.J., Unterwald, E.M., 2009. Cocaine-induced hyperactivity and sensitization are dependent on GSK3β. Neuropharmacology 56, 1116–1123.
Nwaneshiudu, C.A., Unterwald, E.M., 2010. NK-3 receptor antagonism prevents behavioral sensitization to cocaine: a role of glycogen synthase kinase-3 in the nucleus accumbens. J. Neurochem. 115, 635–642.
Paxinos, G., Watson, C., 2004. The Rat Brain in Stereotaxic Coordinates, fourth ed. Elsevier Academic Press.
Perrine, S.A., Miller, J.S., Unterwald, E.M., 2008. Cocaine regulates protein kinase B and glycogen synthase kinase-3 activity in selective regions of rat brain. J. Neurochem. 107, 570–577.
Peineau, S., Taghibiglou, C., Bradley, C., Wong, T.P., Liu, L., Lu, J., et al., 2007. LTP inhibits LTD in the hippocampus via regulation of GSK3β. Neuron 53, 703–717.
Robbins, T.W., Cador, M., Taylor, J.R., Everitt, B.J., 1989. Limbic-striatal interactions in reward-related processes. Neurosci. Biobehav. Rev. 13, 155–162.
Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Brain Res. Rev. 18, 247–291.
Robinson, T.E., Berridge, K.C., 2003. Addiction. Annu. Rev. Psychol. 54, 25–53.
Song, M.J., Jang, J.K., Kim, W.Y., Yoon, H.S., Kim, J.H., 2013. Inhibition of PKMzeta in the nucleus accumbens core blocks the expression of locomotor sensitization induced by amphetamine. Behav. Brain Res. 241, 139–143.
Swortwood, M.J., Carlier, J., Ellefsen, K.N., Wohlfarth, A., Diao, X., Concheiro-Guisan, M., Kronstrand, R., Huestis, M.A., 2016. In vitro, in vivo and in silico metabolic profiling of α-pyrrolidinopentiothiophenone, a novel thiophene stimulant. Bioanalysis 8, 65–82.

Uchiyama, N., Matsuda, S., Kawamura, M., Shimokawa, Y., Kikura-Hanajiri, R., Aritake, K., Urade, Y., Goda, Y., 2014. Characterization of four new designer drugs, 5-chloro-NNEI, NNEI indazole analog, α-PHPP and α-POP, with 11 newly distributed designer drugs in illegal products. Forensic Sci. Int. 243, 1–13.

Vezina, P., 2004. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neurosci. Biobehav. Rev. 27, 827–839.

Wei, J., Liu, W., Yan, Z., 2010. Regulation of AMPA receptor trafficking and function by glycogen synthase kinase 3β. J. Biol. Chem. 285, 26369–26376.

Welter-Luedeke, J., Maurer, H.H., 2016. New psychoactive substances: chemistry, pharmacology, metabolism, and detectability of amphetamine derivatives with modified ring systems. Ther. Drug Monit. 38, 4–11.

Wojcieszak, J., Andrzejczak, D., Woldan-Tambor, A., Zawilska, J.B., 2016. Cytotoxic activity of pyrovalerone derivatives, an emerging group of psychostimulant designer cathinones. Neurotox. Res. 30, 239–250.

Xu, C.M., Wang, J., Wu, P., Zhu, W.L., Li, Q.Q., Xue, Y.X., Zhai, H.F., Shi, J., Lu, L., 2009. Glycogen synthase kinase 3β in the nucleus accumbens core mediates cocaine-induced behavioral sensitization. J. Neurochem. 111, 1357–1368.

Xu, C.M., Wang, J., Wu, P., Xue, Y.X., Zhu, W.L., Li, Q.Q., Zhai, H.F., Shi, J., Lu, L., 2011. Glycogen synthase kinase 3β in the nucleus accumbens core is critical for methamphetamine-induced behavioral sensitization. J. Neurochem. 118, 126–139.