Con formato: Inglés (Estados Unidos)

Association **between the expression of amphetamine-induced behavioral sensitization and Cdk5/p35 activity in Dorsal Striatum**

Con formato: Fuente de párrafo predeter., Fuente: (Predeterminado) Times New Roman, 12 pto, Sin Negrita, Color de fuente: Automático, Español (España, internacional), Diseño: Claro

Con formato: Centrado, Interlineado: 1,5 líneas

~~**Behavioral expression of amphetamine sensitization is mediated by Cdk5/p35**~~

Con formato: Resaltar

Estela Cecilia Mlewski[1], Carlos Arias[1*] and Gabriela Paglini[1*]

Con formato: Español (España, internacional)

[1]Laboratory of Neurobiology, Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET-Universidad Nacional de Córdoba, Córdoba, Argentina.

*These authors shared the last authorship.

Corresponding author: Gabriela Paglini (Ph.D.)

Con formato: Español (España, internacional)

Laboratory of Neurobiology, Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET-Universidad Nacional de Córdoba. Córdoba, Argentina.

Friuli 2434, 5016 - Córdoba Argentina.

Phone: 54-351-4681465 (ext. 133) Fax: 54-351-4695163.

E-mail: gpaglini@immf.uncor.edu

Con formato: Español (España, internacional)

Con formato: Inglés (Estados Unidos)

Con formato: Español (España, internacional)

Abstract

Sensitization to psychostimulants is strongly influenced by the environmental context in which the drug is administered and little is known about the molecular mechanisms that regulate this process. Chronic treatment with psychostimulants has been shown to up-regulate the expression of cyclin-dependent kinase 5 (Cdk5) in the striatum, as a downstream target gene of ΔFosB. This study was therefore designed to analyze neurochemical changes underlying the expression of ~~amph~~amphetamine~~etamine (amph)~~-induced sensitization. To this end, periadolescent rats were given saline or 4 mg/kg ~~amph~~amphetamine in a NOVEL or a HOME environment. After one day ~~of withdrawal~~, subjects were challenged with vehicle or 2 mg/kg ~~amph~~amphetamine in the same context in which they received the first administration of the drug. Locomotor activity and the expression levels of p35 and Cdk5 activity in synaptosomes of the dorsal striatum were analyzed. The expression of behavioral sensitization was observed only in the NOVEL condition. Furthermore, only animals trained and tested in the NOVEL condition showed increased p35 protein levels and Cdk5 activity. Our findings provide clear behavioral and neurochemical evidence of a specific association between increased p35 and Cdk5 activity in the dorsal striatum and the expression of ~~amph~~amphetamine-behavioral sensitization, allowing us to propose p35 as a biochemical marker of behavioral sensitization to ~~amph~~amphetamine.

Con formato: Resaltar

Key words:

~~Ampl~~Amphetamine~~etamine~~; Cdk5/p35; behavioral sensitization; novelty; synaptosomes; two-injection protocol

Con formato: Inglés (Estados Unidos)

1. Introduction

Repeated exposure to psychostimulants results in a progressive and enduring enhancement of the motor stimulant effect elicited by a subsequent drug challenge, a phenomenon known as behavioral sensitization. This phenomenon is proposed to model the increased drug craving observed in human psychostimulant abusers (Robinson and Berridge, 1993, 2003; Vanderschuren and Kalivas, 2000). It is known that psychostimulants may cause long-lasting behavioral changes, partly via stimulation of dopamine D1 receptors and increasing the levels of the transcription factors, cFos, cJun, Zif-268, and ΔFosB in the dorsal and ventral striatum (Ehrlich et al., 2002; Kelz et al., 1999; McClung and Nestler, 2003; Nestler, 2001; Robison et al., 2013). Chronic treatment with ~~coc~~cocaine has been shown to up-regulate the expression of Cyclin-dependent kinase 5 (Cdk5) in the striatum, as a downstream target gene of ΔFosB (Bibb et al., 2001; Kumar et al., 2005). It has also been demonstrated that the induction of Zif-268, through the ERK pathway, increases the expression of the Cdk5 activator protein, p35 (Harada et al., 2001). Cdk5 is a member of the serine/threonine kinases that is activated upon interaction with a regulatory subunit, either p35 or p39, identified in the brain (Humbert et al., 2000; Tang et al., 1995; Tsai et al., 1994) and the level of p35 protein is also known to be a rate-limiting factor for Cdk5 activity (Takahashi et al., 2005). Cdk5/p35 complex has been implicated in multiple brain processes, including neuronal development, dopaminergic signaling and transmission, and also in memory formation (Angelo et al., 2006; Chergui et al., 2004; Hawasli

Con formato: Inglés (Estados Unidos)

et al., 2007; Lai and Ip, 2009; Paglini and Caceres, 2001; Pigino et al., 1997). In addition, dysregulation of Cdk5 has also been implicated in many neurological disorders (Chen and Wang, 2010; Ikiz and Przedborski, 2008; Krapacher et al., 2010). Further, Cdk5 has been demonstrated to dampen the effect of chronic ~~coc~~cocaine administration, indicating a possible regulatory role of this kinase in drug addiction (Bibb et al., 2001). Studies conducted in our laboratory have demonstrated a transient enhanced expression of p25, the truncated fragment of p35, after acute and chronic ~~amph~~amphetamine administration, supporting the idea that the increases in Cdk5 activity could be specifically involved in the cellular events underlying sensitization to psychostimulant drugs (Mlewski et al., 2008), but up to date, this association has not been wholly demonstrated.

It is well known that context novelty plays an important role in the development of locomotor sensitization to psychostimulants (Anagnostaras and Robinson, 1996; Ferguson et al., 2004; Stewart and Badiani, 1993). The fact that the circumstances surrounding drug administration strongly modulate the expression of behavioral sensitization gives us an excellent opportunity to study some mechanisms by which drugs of abuse exert both short and long-lasting effects on the brain (Badiani and Robinson, 2004).

Although data from our laboratory, and others, have shown that chronic exposure to psychostimulants up-regulates the expression levels of the Cdk5/p35 complex, no studies have revealed its association with the behavioral sensitization process. The present study was therefore designed to explore the possible specific association between Cdk5/p35 complex and the expression of ~~amph~~amphetamine-induced sensitization. To this end, we generated two experimental conditions, NOVEL and HOME context, that differentially promoted the expression of behavioral sensitization, and we took advantage of the sensitivity of the two-

injection protocol (Valjent et al., 2010) to detect specific alterations in signaling mechanisms, compared with repeated injection ~~paradigms~~procedures. Since it is not necessary to repeatedly administer a psychostimulant drug to produce behavioral sensitization. A single injection of cocaine ~~(coc)~~ or ~~amph~~amphetamine~~etamine (amph)~~ can promote this effect (i.e. enhanced locomotor activity), which is revealed by a second injection of the same or another drug given hours, days, or even weeks after the first administration (Alvarez Jdo et al., 2006; Chinen et al., 2006; Jackson and Nutt, 1993; Robinson et al., 1982; Vanderschuren et al., 1999). This protocol cannot be compared with other ~~paradigm~~procedures because repeated injections of drugs can trigger biochemical responses that are different from those induced by single exposures, making it very difficult to establish causality links between behavioral and biochemical responses (Valjent et al., 2010). Besides, the use of this ~~paradigm~~procedure provides interesting clues about the role of specific signaling pathways *in vivo* (Corbille et al., 2007; Paz et al., 2013; Stipanovich et al., 2008; Valjent et al., 2005) and offers an excellent model for the molecular dissection of the long-lasting effects of drugs of abuse (Kameda et al., 2011; Vanderschuren and Kalivas, 2000; Vezina and Leyton, 2009).

The present study was conducted with adolescent rats. Although most of the studies focused on cellular and molecular changes associated with psychostimulants are conducted with adult organisms, we consider it important to perform studies in adolescents, given the fact that in this ontogenetic phase the subjects are particularly vulnerable to the development of drug addiction (Spear 2000; Kameda et al., 2011), and that some studies suggest that adolescents are more predisposed to display behavioral sensitization to stimulants than adults (Laviola, 2003; Kameda et al., 2011; Mathews et al., 2011).

Con formato: Inglés (Estados Unidos)

~~Furthermore, we consider important to perform the experiments in adolescent rats given the fact that in this ontogenetic phase the organisms are particularly vulnerable to the development of drug addiction (Spear 2000; Kameda et al., 2011) and little is known about the cellular and molecular mechanisms underlying the effects of stimulants drugs in relevant brain areas in this developmental stage.~~

Con formato: Inglés (Estados Unidos)

Con formato: Sin Resaltar

2. Materials and Methods

2.1. Animals

Periadolescent male Wistar rats (33-35-days-old) of our breeding, weighing approximately 120g at the onset of the experiment, were housed in standard laboratory Plexiglas cages in a climate-controlled room at 22 ± 1°C with a 12-h dark and 12-h light cycle, with free access to food and water. We used 64 male rats for the behavioral experiment (n= 8 per group), and 128 male rats for biochemical analyses (n= 8 per group). All the experiments were carried out during the light cycle, between 10:00 am and 3:00 pm, in a separate behavioral testing room. During the test, the room was quiet and dimly lit, and rats were allowed to acclimatize to the room for 30 min before the start of each experiment. All animal care and experimental procedures were approved by the National Department of Animal Care and Health (SENASA - ARGENTINA) and were in compliance with the National Institute of Health general guidelines for the Care and Use of Laboratory Animals. Efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

Con formato: Inglés (Estados Unidos)

D-~~amph~~amphetamine~~etamine~~ sulfate (Parafarm, Switzerland~~, amph~~) was dissolved in sterile saline (NaCl 0.9%, sal). Animals were intraperitoneally (i.p.) injected at doses of 0 (vehicle only), 2 or 4 mg/kg of ~~amph~~amphetamine. Dosage of D-amphetamine was selected on the basis of a prior study from our laboratory in which we observed behavioral sensitization with these doses (Mlewski et al., 2008).

2.3. Behavioral assessment

Following maternal separation, rats were housed in pairs (2 per cage, HOME cage) and maintained in this way until the end of the experiment. The experiment began when the rats reached an age of 33 days. One of the two rats in the HOME cage went to a cylindrical (40 cm diameter), open field (NOVEL condition) and the other rat remained in the home cage (HOME condition) where it received all experimental treatments.

Drug-induced locomotor activity was evaluated using a two-injection protocol, which involved both, a "Training" phase (first injection on day 1) and a "Challenge" phase (test day, second injection).

On the first day (Training), animals received one injection of vehicle (sal) or ~~amph~~amphetamine (4 mg/kg). After the injection, the rats were immediately placed in the corresponding test cage, which was either HOME (the cage where the rat was housed since maternal separation) or NOVEL (open field)] and locomotor activity was recorded every 5 minutes, for a total session lasting 1 hour.

The Challenge was conducted 24 hours after the Training phase ~~a drug withdrawal period of 1 day~~ (on the third day of the experiment). In this phase, half of the subjects treated with ~~amph~~amphetamine and half of those treated with sal during the Training received 2mg/kg

Con formato: Inglés (Estados Unidos)

~~amph~~amphetamine, while the remaining rats received sal. After the Challenge injection, they were immediately placed in the same environment where they were evaluated during the Training day. Consequently, there were 4 groups for the HOME condition (sal/sal, sal/~~amph~~amph, ~~amph~~amph/sal and ~~amph~~amph/~~amph~~amph) and the same number of groups for the NOVEL condition.

We assessed locomotor activity by counting the number of quadrants that were crossed by the animal. The floor of the NOVEL and HOME environments was divided into 4 quadrants. A trained researcher blind to the experimental conditions registered the number of quadrants crossed (head and front member for one score) during the test.

2.4. Biochemical analysis

Another set of rats was behaviorally evaluated and killed by decapitation at either 1 h or 4 h after the Challenge. Brains were rapidly removed and chilled in ice-cold sal. The dorsal striatum was dissected over ice and subjected to a subcellular fractionation technique and Cdk5 activity assay.

2.4.1. Preparation of synaptosome fractions

Procedures for preparing crude synaptosome fractions were performed as described previously, with minor modifications (De Camilli et al., 1983). Briefly, the tissue was homogenized in 20 volumes of ice-cold sucrose buffer/g original tissue (0.32 M sucrose, 10mM HEPES and 1 mM EDTA, pH 7.4, 1 µg/ml Aprotinin, 1 µg/ml Leupeptin, 100 µg/ml PMSF, 1 µg/ml Pepstatin, and 0.2 mM Sodium Orthovanadate), using a small Potter glass–Teflon homogenizer and centrifuged at 1000 x g for 10 min. The supernatant (total homogenate) was then centrifuged at 10000 x g for 20 min to obtain the cytosolic fraction and the resulting pellet

Con formato: Inglés (Estados Unidos)

was resuspended in cold lysis buffer RIPA (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 µg/ml Aprotinin, 1 µg/ml Leupeptin, 100 µg/ml PMSF, 1 µg/ml Pepstatin, and 0.2 mM Sodium Orthovanadate) to obtain a crude synaptosomal fraction (SF) (Mlewski et al., 2008). Only the SF was used for western blot analysis and Cdk5 kinase activity assay.

2.4.2. Western blot analysis

SFs from individual rats were assayed for protein determination (DC protein assay Kit, Bio-Rad laboratories). Equal amounts of protein (20 µg) were separated by 12% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to PVDF membranes (Amersham Biosciences, Buckinghamshire, England). Membranes were then blocked in 5% skim milk in TBST (10 mM Tris, pH 7.5, 150 mM NaCl plus 0.05% Tween-20) for 1h, and then incubated overnight at 4ºC with their primary antibodies diluted in TBST. Polyclonal antibody anti-p35 (1:400, C-19, Santa Cruz Biotechnology) and anti-α-tubulin (1:3000, DM1A, Sigma-Aldrich) were used. Membranes were then washed three times in TBST and incubated with a secondary horseradish peroxidase-conjugated antibody (1:2000, donkey anti-Rabbit IgG-HRP and 1:2000 donkey anti-Mouse IgG-HRP from Jackson Immunoresearch Laboratory, West Grove, PA, USA) for 1h at room temperature. After five washes with TBST, the blots were developed using an enhanced chemiluminescence detection kit (ECL, Amersham Life Science, Buckinghamshire, England). Chemiluminescence was detected by autoradiography using Agfa medical X-Ray Film (Bs.As., Argentina). Densitometry analyses of western blot bands were quantified on scanned films by Scion Image for Windows (Scion Corporation Frederick, MD, USA) analysis software.

Con formato: Inglés (Estados Unidos)

Values of the proteins of interest were normalized to α-tubulin and the ratios were then used to perform statistical analysis.

2.4.3. Cdk5 kinase activity assay

For in vitro Cdk5 activity kinase assays, 250 µg of total cellular proteins from SF lysates were immunoprecipitated with anti-Cdk5 antibody conjugated to agarose beads (C-8, Santa Cruz Biotechnology) as described previously (Mlewski et al., 2008). Briefly, immunoprecipitates were washed three times with lysis buffer RIPA and once with kinase buffer (30 mM MOPS, pH 7.2, 5 mM MgCl2, and 0.1 mM cold ATP). The washed beads were then incubated with kinase buffer containing 1.5 µg of Histone H1 (Roche Diagnostics Corporation) as Cdk5 substrate, and 5 µCi of [γ-32P] ATP in a final volume of 30 µl. After 30 min of incubation at 30ºC, 10 µl of 4x Laemmli sample buffer (250 mM Tris-HCL, 40% glycerol, 10% SDS, 10% 2-mercaptoethanol and 0,1% bromophenol blue at ph 6.8) was added to each sample to stop the reaction and they were then analyzed by SDS-PAGE using 12% polyacrylamide gels. Once the gels were dried, radiographic bands were visualized in a phosphoimage instrument (Storm 840, Molecular Dynamics) and quantified using the Image Quant software (Molecular Dynamics).

2.5. Design and Statistical analysis

The factorial design for the HOME and NOVEL behavioral experiment was defined by ~~amph~~amphetamine (amph) treatments in the Training (0 or 4 mg/kg) and Challenge (0 or 2 mg/kg) phase. The design resulted in four independent groups: sal/sal, sal/~~amph~~amph, ~~amph~~amph/sal and ~~amph~~amph/~~amph~~amph. Behavioral data for both HOME and NOVEL conditions were analyzed separately by means of two-way mixed ANOVAs: 2 [Treatment on

Training (sal or ~~amph~~amph)] X 2 [Treatment on Challenge (sal or ~~amph~~amph)]. Results of locomotor activity were expressed as total mean values +/- SEM. In order to make specific group comparisons, post-hoc Duncan's multiple range tests were performed.

Biochemical data were analyzed by a one-way ANOVA followed by post-hoc Tukey analysis to enable specific group comparison. The level of statistical significance was set at $p<0.05$. All the data were analyzed using the Statistic 5.1 program (Statsoft, Inc.; OK, USA).

3. Results

3.1. Expression of sensitization as a function of context novelty in a two-injection protocol

~~We have previously shown that adolescent rats increased locomotion response to 4 mg/kg of amphetamine chronically administrated and when challenged with the half dose (2 mg/kg) after two days of withdrawal, those rats pre-treated with amphetamine exhibited a significant increase in locomotor activity compared with the saline-treated group during the training (Mlewski, et al 2008).~~ç~~Considering ~~Given~~ that novelty is known to play a critical role in behavioral sensitization, we ~~developed conducted~~used an experimental setting to manipulate the expression of behavioral sensitization. To this end, the two-injection protocol was applied in a familiar (HOME condition) and in a novel environment (NOVEL condition), as described in section 2.3

Figure 1A shows total activity scores in the HOME environment, during both the Training (first injection) and Challenge (second injection) trials. The ANOVA revealed a significant main effect of ~~amph~~amphetamine treatment at Training, $F(1,29)= 7.415$, indicating that animals given 4 mg/kg ~~amph~~amphetamine (groups ~~amph~~amph/sal and ~~amph~~amph/~~amph~~amph) moved more than sal-treated controls (groups sal/sal and

Con formato: Inglés (Estados Unidos)

Con formato: Resaltar

Con formato: Inglés (Estados Unidos)

sal/~~amph~~amph). An ANOVA conducted on the data from the challenge trial found a significant main effect of ~~amph~~amphetamine at Challenge [$F(1,29)= 8.856$; $p<0.01$], indicating that, regardless of the training experience, rats treated with ~~amph~~amphetamine (2 mg/kg; groups sal/~~amph~~amph and ~~amph~~amph/~~amph~~amph) displayed higher locomotor activity scores than those treated with sal.

As expected for this HOME condition, animals that received ~~amph~~amphetamine for the second time (group ~~amph~~amph/~~amph~~amph) showed no statistical difference in comparison with those that received ~~amph~~amphetamine for the first time (group sal/~~amph~~amph), thereby indicating that ~~amph~~amphetamine treatment in the HOME condition was not sufficient to induce locomotor sensitization.

"**Figure 1** about here"

The remaining half of the subjects were evaluated in the NOVEL condition. Figure 1B shows total locomotor activity scores of these subjects during both the Training and Challenge phases. The ANOVA revealed a significant main effect of ~~amph~~amphetamine treatment at Training [$F(1,29)= 149.41$; $p<0.001$], indicating that subjects treated with 4 mg/kg ~~amph~~amphetamine exhibited higher locomotor activity scores than sal-treated subjects. The ANOVA conducted with activity data collected during the Challenge indicated a significant main effect of ~~amph~~amphetamine at Challenge [$F(1,29)= 53.34$; $p<0.001$] and a significant interaction between ~~amph~~amphetamine treatment at Training and at Challenge [$F(1,29)= 6.57$; $p<0.05$]. According to the post-hoc analyses, locomotor activity scores from ~~amph~~amph/~~amph~~amph and

sal/~~amph~~amph groups were significantly higher than those from their respective sal control conditions.

Interestingly, and relevant to the aims of the study, when challenged with ~~amph~~amphetamine the animals pretreated with ~~amph~~amphetamine during the training phase (~~amph~~amph/~~amph~~amph group) displayed significantly higher locomotor activity than those pretreated with sal (sal/~~amph~~amph group), thereby indicating that behavioral sensitization to the stimulating effect of ~~amph~~amphetamine had been expressed. There were no statistical differences between groups treated with sal at the Challenge phase (groups: sal/sal and ~~amph~~amph/sal). These results demonstrate that, in the NOVEL condition, the two-injection protocol using 4 mg/kg of ~~amph~~amphetamine and challenging with the half dose induced locomotor behavior sensitization. These behavioral data, obtained by combining the two-injection protocol with the manipulation of the circumstances surrounding ~~amph~~amphetamine administration, provided us with an excellent tool to investigate short-term neurochemistry modifications necessary to evoke long-lasting adaptations underlying behavioral sensitization.

3.2. Expression and activity of Cdk5/p35 associated with the expression of locomotor sensitization to ~~amph~~amphetamine in a two-injection protocol

It is well known that the striatum plays an important role in locomotor activity and reward-related behaviors, and psychostimulants drugs cause long- and short-term behavioral and biochemical changes in the dorsal and ventral striatum (Nestler, 2013). Previous data obtained from acute and chronic amphetamine-administered rats showed a raised and lasting expression of p35 protein level in dorsal striatal synaptosomal fraction (SF) of periadolescent rats, and this increase can be observed 4 hours after the amphetamine treatment and last to at least 24 hours.

Con formato: Inglés (Estados Unidos)

Con formato: Fuente: Times New Roman, 12 pto

Con formato: Sangría: Primera línea: 1,25 cm

Moreover, p25 protein (proteolytic fragment of p35) increased its expression 4 hours after the last amph- injection, returning to the basal level after 24 hours, indicating that this rapid modulation of this protein could play a role during the expression of the sensitized response (Mlewski et al., 2008). ~~It is well known that the striatum plays an important role in processing information related to motor function and reward-related behaviors and psychostimulants drugs cause long and short behavioral and biochemical changes in the dorsal and ventral striatum (Nestler, 2013). Previous data obtained from acute and chronic amphetamine-administered rats showed a raised and lasting expression of p35 protein level since 4 hours to at least 24 hours post last amphetamine-administration, in dorsal striatal synaptosomal fraction (SF) of periadolescent rats. Moreover, p25 protein (proteolytic fragment of p35) increased its expression 4 hours after the last amphetamine- injection, returning to the basal level after 24 hours, indicating that this rapid modulation of this protein could play a role during the expression of the sensitized response (Mlewski et al., 2008).~~

In order to analyze a specific association between p35 and the expression of sensitization, we examined the expression levels of this protein in the ~~synaptosomal fraction (~~SF~~)~~ of the dorsal striatum of animals from the HOME and NOVEL conditions, sacrificed immediately after finished the testing session (1 hour) ~~(Fig. 2A)~~ or 4 hours ~~(Fig. 2B)~~ after the ~~amph~~amphetamine Challenge.

Western blot analysis on striatal SF from HOME condition animals showed that amphetamine had any effect on the expression of p35 levels; neither 1 hour (Fig. 2A) nor 4 hours ~~The~~ (Fig. 2B) after Challenge. The ANOVA did not detect significant effects or interactions at any of these time points, indicating that neither the increase in locomotor activity induced by

Con formato: Inglés (Estados Unidos)

Con formato: Fuente: 12 pto

~~amph~~amphetamine during the Challenge day, nor the prior experience with ~~amph~~the drug on the Training day modified the expression of p35 in the dorsal striatum in a familiar environment.

Con formato: Inglés (Estados Unidos)

Con formato: Inglés (Estados Unidos)

Con formato: Interlineado: Doble

"**Figure 2** about here"

Figure 3 shows p35 levels in the dorsal striatum at 1 hour (A) and 4 hours (B) after ~~amph~~amphetamine Challenge in the NOVEL condition. In this case, the statistical analysis revealed a biochemical pattern similar to the behavioral profile. The data analyses showed that, at both of the times tested, p35 was only increased in ~~amph~~amphetamine challenged animals that had received prior experience with ~~amph~~amphetamine in the Training phase. The ANOVA found a significant interaction between ~~amph~~amphetamine in the Training phase and ~~amph~~amphetamine in Challenge [$F(1,44)=5.67$, $p<0.05$]. In addition, post-hoc analyses showed an increase in p35 levels only in the ~~amph~~amph/~~amph~~amph group compared with the remaining groups (~~amph~~amph/sal or sal/~~amph~~amph). This effect was observed regardless of the time point at which samples were collected (1 or 4 hours after ~~amph~~amphetamine Challenge treatment).

"**Figure 3** about here"

Results from *in vivo* studies have demonstrated that p35 is the rate-limiting factor for the regulation of Cdk5 activity (Takahashi et al., 2005). For this reason, we attempted to determine whether the increase in p35 in the NOVEL context correlates with an increase in Cdk5 activity. To this end, we examined Cdk5 kinase activity in animals trained and challenged in the NOVEL

context. As we expected, IP of Cdk5 followed by kinase activity assays using histone H1 as a substrate, revealed that Cdk5 activity increased dramatically in the SF of sensitized rats (group ~~amph~~amph/~~amph~~amph) at both testing times, 1 hour [F(1,12)=7.55, p<0.05] (Fig 3C) and 4 hours [F(1,12)=9.77, p<0.01] (Fig 3D) after ~~amph~~amphetamine Challenge treatment.

Discussion

~~B.F. Singer et al. / Neuropharmacology 85 (2014) 243e252~~
~~NAcc roscovitine (Ros) administered during exposure blocked the induction of conditioned locomotion but spared the induction of locomotor sensitization.~~
~~The present findings indicate that perturbing Cdk5 and Kal7 signaling in the NAcc exclusively during exposure to amphetamine prevents the development of conditioned locomotion but spares the development of locomotor sensitization by the drug. Therefore, Cdk5 and Kal7 signaling in the NAcc is necessary for the induction of excitatory contextual associative conditioning but not for nonassociative forms of plasticity such as sensitization.~~

~~B_F_ _S_i_n_g_e_r_ _e_t_ _a_l_._ _/_ _B_e_h_a_v_i_o_u_r_a_l_ _B_r_a_i_n_ _R_e_s_e_a_r_c_h_ _2_7_5_ _(_2_0_1_4_) _9_6_–_1_0_0_ _~~

~~A b s t r a c t When psychostimulant drugs like amphetamine are administered repeatedly in the presence of a contex-tual stimulus complex, long-lasting associations form between the unconditioned effects of the drug and the contextual stimuli. Here we assessed the role played by the proline-directed serine/threonine kinase cyclin-dependent kinase 5 (Cdk5) in the nucleus accumbens (NAcc) on the expression of the conditioned locomotion normally observed when rats are returned to a context previously paired with amphetamine. Infusing the Cdk5 inhibitor roscovitine (40 nmol/0.5 _l/side) into the NAcc 30-min before the test for conditioning significantly enhanced the conditioned locomotor response observed in rats previously administered amphetamine in the test environment. This effect was specific to the expression of a con-ditioned response as inhibiting Cdk5 produced no effect in control rats previously administered saline or previously administered amphetamine elsewhere. As inhibiting Cdk5 during exposure to amphetamine has~~

Con formato: Inglés (Estados Unidos)

Con formato: Inglés (Estados Unidos)

Con formato: Fuente: 10 pto, Inglés (Estados Unidos)

Con formato: Inglés (Estados Unidos)

~~been found to block the accrual of locomotor conditioning, the present results suggest distinct roles for NAcc Cdk5 in the induction and expression of excitatory conditioning by amphetamine.~~

~~In the present experiment, pharmacologically inhibiting Cdk5 inthe NAcc with Ros exclusively on the test for conditioning enhancedthe conditioned locomotor response observed in Paired rats. Thiseffect was specific to the expression of the excitatory conditionedresponse as NAcc Ros produced no significant effects in Unpairedand Control rats.~~

The current study was designed to explore the specific association between the expression of behavioral sensitization induced by ~~amph~~amphetamine and the activity and expression of the Cdk5/p35 complex. The effect of our experimental manipulations, in combination with the two-injection protocol, which induced sensitization in a NOVEL but not in a HOME environment, were strong enough to associate specific changes in Cdk5 activity and p35 levels with this process. Therefore, adolescent rats developed and expressed locomotor sensitization with 4 mg/kg of ~~amph~~amphetamine in a two-injection protocol, but only when they were trained and evaluated in a NOVEL environment. However, the equivalent ~~amph~~amphetamine treatment and manipulation failed to generate such an effect in the HOME condition. More importantly, our results demonstrated that the increased Cdk5 activity and p35 protein levels were only observed in the dorsal striatum of those animals that displayed locomotor sensitization to ~~amph~~amphetamine, suggesting that mere exposure to the drug is not enough to induce changes in the activity and expression levels of these proteins. Taken together, our findings provide clear behavioral and neurochemical evidence of a specific association between increased p35 and Cdk5 activity in the dorsal striatum and the expression of ~~amph~~amphetamine-behavioral

sensitization in a two-injection protocol. Therefore we propose the protein p35 as a possible biochemical marker of psychostimulants behavioral sensitization.

Cdk5 and p35 has been involved in chronic administration of ~~coc~~cocaine (Bibb et al., 2001), meth~~amph~~amphetamine~~etamine~~ (Chen and Chen, 2005), and ~~amph~~amphetamine (Mlewski et al., 2008). Our results revealed that it is not necessary to administer repeated injections of ~~amph~~amphetamine in order to up-regulate these proteins. The two-injection ~~amph~~amphetamine treatment in the NOVEL context was sufficient to generate changes at the level of expression of p35 and Cdk5 activity. In support of these findings was the observation that both control conditions (groups: sal/~~amph~~amph and ~~amph~~amph/sal) of the NOVEL context, and animals trained and challenged in the HOME environment, failed to show any changes in p35 protein levels. It is important to point out that this is the first study in which an environmental condition (HOME or NOVEL) combined with the two-injection protocol has revealed a direct association between sensitization and increased p35 protein levels. Further, the elevated activity of Cdk5 may be a result of the increased protein level of the Cdk5 co-activator p35, as previously demonstrated *in vivo* (Takahashi et al., 2005). Thus, our data suggest that, irrespective of the administration protocol employed, increased p35 levels in the dorsal striatum is required to activate Cdk5 kinase ~~—~~ an observed ~~n essential~~ condition ~~for~~of ~~the~~ expression of locomotor sensitization induced by ~~amph~~amphetamine.

~~1.~~ Two recent studies suggest that Cdk5 activity would not necessarily be implicated in the development of amphetamine-sensitization. Singer et al. (2014 a and b) observed that inhibition of Cdk5 activity with roscovitine interfered with Pavlovian conditioning acquired during the exposure to amphetamine. Their results suggest that Cdk5 may play opposing roles

Con formato: Inglés (Estados Unidos)

Con formato: Espacio Después: 0 pto, Interlineado: Doble

Con formato: Espacio Después: 0 pto, Interlineado: Doble

during the acquisition and expression of associative learning, since roscovitine prevented conditioning when it was given during the training phase, and enhanced the conditioned response when it was administered soon before testing. Interestingly, while interfering with Pavlovian conditioning, rRoscovitine administration did not affect sensitization induced by amphetamine. Considering these results, it is plausible that the increase in Cdk5/p35 activity that we observed in the NOVEL, but not in the HOME condition, is linked to contextual Pavlovian conditioning induced by amphetamine, and not with the expression of sensitization. This possibility is congruent with the fact that sensitization induced by the two-injection protocol can involve contextual learning (Valjent, 2010). In the present study, we do not present additional data that might allow us to infer the role that Cdk5 may be playing, but we can contrast our results with the existing evidence. Firstly, it is important to note that, unlike Singer et al. found, our protocol did not result in a conditioned response in the NOVEL condition. This effect would give rise to an increase in locomotor activity at testing in the amph/sal group when compared to the sal/sal group, which was not observed. Furthermore, we did not find significant differences between these groups when levels of expression of p35 or activity of Cdk5 were examined. The function that Cdk5 is playing in the behavioral response to psychostimulants is unclear, since inhibition of Cdk5 in the ventral striatum can result in inhibition (Chen and Chen, 2005), potentiation (Bibb et al., 2001; Taylor et al., 2007) or even have no effect (Singer et al., 2014 a and b) upon the locomotor sensitized response induced by psychostimulants. Differences in the structure (dorsal vs ventral striatum) and the protocols used, including the type of drug, may account for this variability. The main contribution of our study to this field is to point out one environmental variable that may jointly modulate the activity of the Cdk5/p35 complex in the dorsal striatum and the expression of sensitization induced by amphetamine. ~~Two recent studies suggest that~~

Con formato: Inglés (Estados Unidos)

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Cdk5 activity in the NAcc after amphetamine exposure would not necessarily be implicated in the development of amphetamine sensitization. Singer et al. (2014 a and b) observed that inhibition of Cdk5 activity with rRoscovitine interfered with Ppavlovian conditioning acquired during the exposure to amphamphetaminecetamine. Their results suggest that Cdk5 may play an opposite role during the acquisition than during the expression of associative learning, since rRoscovitinea prevented conditioning when it was given during the training phase, and enhanced the conditioned response when it was administered soon before testing. Interestingly, while interfering with Ppavlovian conditioning, Roscovitine administration did not affect sensitization induced by amphamphetaminecetamine. Considering these results, it is plausible that the increase in Cdk5/p35 activity that we observed in the novelNOVEL, but not in the home HOME, condition, is linked to contextual Ppavlovian conditioning induced by amphamphetaminecetamine, and not with the expression of sensitization. This possibility is congruent with the fact that sensitization induced by the two- injection protocol injection can involve contextual learning (Valjent, 2010). In the present study, we do not present additional data that allow inferring the role that Cdk5 may be playing, but we can contrast our results with the existing evidence. Firstly, it is important to note thatn, unlike Singer et al. found, our protocol did not result in a conditioned response in the novel NOVEL condition. This effect would give place to an increase in locomotor activity at testing in the Amphamph/ sSal group when compared to the sSal/s Sal group, which was not observed. Furthermore, we did not find significant differences between these groups when levels of expression of p35 or activity of Cdk5 were examined. The function that Cdk5 is playing in the behavioral response to psychostimulants is unclear, since iInhibition of Cdk5 in the ventral striatum can result in inhibition (Chen and Chen, 2005Chen), potentiation (Bibb et al., 2001; Taylor et al., 2007, Bibb)

Con formato: Inglés (Estados Unidos)

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

Con formato ...

~~or even be ineffective (Singer et al., 2014 a and b) upon the locomotor sensitized response induced by psychostimulants. Differences in the structure (dorsal vs ventral striatum) and the protocols used, included the type of drug, may account this variability. The main contribution of our study to this field is to point out into one environmental variable that may jointly modulate the activity of the Cdk5/p35 complex in the dorsal striatum and the expression of sensitization induced by amphetamine.~~

~~Cdk5, although as mentioned, changes in Cdk5 activity may not be necessarily related with sensitization induced by amphetamine or cocaine, at least in the NAcc (Singer et al., 2014; Seiwell et al., 2007).~~

~~literatura conflictiva~~

Whilst it was not an aim of the present study to identify the target substrates at which this increased Cdk5 activity is directed, it has been reported that the Cdk5/p35 complex exerts a regulating effect on the dopamine pathway after several days of cocaine administration (Bibb et al., 2001). In this particular administration protocol, Valjent and colleagues (Valjent et al., 2010) have characterized many of the biochemical changes underlying some mechanisms of locomotor sensitization to cocaine in a two-injection protocol, wherein the dopaminergic pathway is clearly involved, and required the stimulation of both dopamine D1 (DR1) and glutamate (NMDA) receptors, as well as ERK activation and novo protein synthesis. With respect to this issue, it has been shown that DR1 and dopamine D2 (DR2) receptors play a critical role in the locomotor

Con formato: Inglés (Estados Unidos)

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Color de fuente: Automático, Inglés (Estados Unidos), Diseño: Claro

Con formato: Fuente: (Predeterminado) Times New Roman, 12 pto, Inglés (Estados Unidos)

Con formato: Interlineado: Doble

Con formato: Inglés (Estados Unidos)

Con formato: Sangría: Primera línea: 1,25 cm

Con formato: Inglés (Estados Unidos)

effects of amphetamine and cocaine, whereas DR1 seem to be involved in the development of amphetamine sensitization, and DR2 in its expression (Dias et al., 2004; Vanderschuren and Kalivas, 2000). Strikingly, D2R has recently been identified as a novel substrate of Cdk5, in the striatopallidal D2R medium spiny neuron population. This interaction suggests a new regulatory mechanism of the dynamic nature of D2R surface availability (Jeong et al., 2013). Given the fact that we used amphetamine in our experimental design, and DR2 receptors have been involved in the expression of locomotor sensitization, it would be reasonable to think that the increased Cdk5/p35 activity observed in this study may also be directed to regulate the dynamic process of surface receptor availability. Whilst this provides further insight into the adaptive changes of the dopamine system in response to drug exposures, implicating a role for Cdk5, further research is needed to explore this possibility.

Although it has been shown that it is the long-lasting qualities of behavioral sensitization that make it such a potentially important component of drug addiction, it is accepted that short-term transient modifications are necessary to evoke long-lasting adaptations that mediate persistent drug hyperresponsiveness (Vanderschuren and Kalivas, 2000; Wolf and Xue, 1998). The present data are compatible with the idea that the activation of Cdk5/p35 complex in striatum may be an important step in the cascade of molecular events linked to a single experience with psychostimulants, although as discussed, the role of the Cdk5/-p35 complex may be related to different learning processes occurring during experience with the drug.~~Whilst it was not an aim of the present study to identify the target substrates at which this increased Cdk5 activity is directed, it has been reported that the Cdk5/p35 complex exerts a regulating effect on the dopamine pathway after several days of coccocaine administration (Bibb et al., 2001). In this particular administration protocol, Valjent and colleagues (Valjent et al., 2010) have~~

Con formato: Inglés (Estados Unidos)

characterized many of the biochemical changes underlying some mechanisms of locomotor sensitization to coccocaine in a two-injection protocol, wherein the dopaminergic pathway is clearly involved, and required the stimulation of both dopamine D1 (DR1) and glutamate (NMDA) receptors, as well as ERK activation and novo protein synthesis. With respect to this issue, it has been shown that DR1 and dopamine D2 (DR2) receptors play a critical role in the locomotor effects of amphamphetamine and coccocaine, whereas DR1 seem to be involved in the development of amphamphetamine sensitization, and DR2 in its expression (Dias et al., 2004; Vanderschuren and Kalivas, 2000). Strikingly, D2R has recently been identified as a novel substrate of Cdk5, in the striatopallidal D2R medium spiny neuron population. This interaction proposes a new regulatory mechanism of the dynamic nature of D2R surface availability (Jeong et al., 2013). Given the fact that we used amphamphetamine in our experimental design, and DR2 receptors have been involved in the expression of locomotor sensitization, it would be reasonable to think that the increased Cdk5/p35 activity observed in this study may also be directed to regulate the dynamic process of surface receptors availability. Whilst this provides further insight into the adaptive changes of the dopamine system in response to drug exposures, implicating a role for Cdk5, further research is needed to explore this possibility.

Although it has been shown that it is its long-lasting qualities that make behavioral sensitization such a potentially important component of drug addiction, it is accepted that short-term transient modifications are necessary to evoke long-lasting adaptations that mediate persistent drug hyperresponsiveness (Vanderschuren and Kalivas, 2000; Wolf and Xue, 1998). The present data support the idea that the activation of Cdk5/p35 complex in striatum may be an important step in the cascade of molecular events ultimately leading to the emergence of a psychostimulant sensitization response. Furthermore, an improved comprehension of the

molecular processes that contribute to the manifestation of behavioral sensitization to psychostimulants is highly relevant for the understanding of development of addiction and places p35 protein as a putative biochemical marker of expression of behavioral sensitization to psychostimulants drugs. Discovery of disruptions in the fine balance that normally exists between brain circuits underling reward, motivation, memory and craving may facilitate the development of new interventions for prevention and treatment of addictive disorders.

The present study suggests that the Cdk5/p35 complex may play a role in the neuroadaptations that underlie various (and as yet poorly understood) aspects of the development of addiction and places p35 protein as a putative biochemical marker of expression of behavioral sensitization to psychostimulants drugs. Discovery of disruptions in the fine balance that normally exists between brain circuits underling reward, motivation, memory and craving have important implications for designing new interventions for the prevention and treatment of addictive disorders.

Con formato: Inglés (Estados Unidos)

Con formato: Inglés (Estados Unidos)

Acknowledgements

This work was supported by grants from FONCyT (PICT 05-38084 and PICT 07-2168). The authors would like to thank Laura Vivas, Dr. Analía Reines and Dr. Anahí Bignante for helpful discussion and criticism, and Dr. Victor Molina for his support and suggestions. We would particularly like to thank the other lab members: F Krapacher, MV Pisano, S Ferreras and AC Solari, and the vivarium technicians for their assistance.

Authors contribution

All authors were responsible for the design of the study. ECM and CA collected animal data and conducted statistical data analysis. ECM performed biochemical essays. ECM, CA and GP interpreted the findings and drafted the manuscript. CA and GP provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved the final version for publication.

Con formato: Inglés (Estados Unidos)

Con formato: Inglés (Estados Unidos)

Con formato: Normal (Web), Espacio Después: 12 pto, Alineación de fuente: Línea de base, Diseño: Claro (Blanco)

Con formato: Inglés (Estados Unidos)

References

Con formato: EndNote Bibliography, Izquierda, Sangría: Izquierda: 0 cm, Sangría francesa: 1 cm, Espacio Antes: 6 pto, Después: 6 pto

~~SPEAR L.P. 2000. The adolescent brain and age-related behavioral manifestations. Neurosci. Biobehav. Rev. 24: 417-463.~~

Alvarez Jdo N, Fukushiro DF, Tatsu JA, de Carvalho EP, Gandolfi AC, Tsuchiya JB, Carrara-Nascimento PF, Lima ML, Bellot RG, Frussa-Filho R (2006) ~~Amph~~Amphetamine~~etamine~~-induced rapid-onset sensitization: role of novelty, conditioning and behavioral parameters. Pharmacology, biochemistry, and behavior 83:500-507.

Anagnostaras SG, Robinson TE (1996) Sensitization to the psychomotor stimulant effects of ~~amph~~amphetamine~~etamine~~: modulation by associative learning. Behavioral neuroscience 110:1397-1414.

Angelo M, Plattner F, Giese KP (2006) Cyclin-dependent kinase 5 in synaptic plasticity, learning and memory. Journal of neurochemistry 99:353-370.

Badiani A, Robinson TE (2004) Drug-induced neurobehavioral plasticity: the role of environmental context. Behavioural pharmacology 15:327-339.

Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, Greengard P (2001) Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 410:376-380.

Chen J, Wang ZF (2010) [Roles of cyclin-dependent kinase 5 in central nervous system development and neurodegenerative diseases]. Sheng li xue bao : [Acta physiologica Sinica] 62:295-308.

Chen PC, Chen JC (2005) Enhanced Cdk5 activity and p35 translocation in the ventral striatum of acute and chronic methamphamphetaminetamine-treated rats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 30:538-549.

Chergui K, Svenningsson P, Greengard P (2004) Cyclin-dependent kinase 5 regulates dopaminergic and glutamatergic transmission in the striatum. Proceedings of the National Academy of Sciences of the United States of America 101:2191-2196.

Chinen CC, Faria RR, Frussa-Filho R (2006) Characterization of the rapid-onset type of behavioral sensitization to amphamphetaminetamine in mice: role of drug-environment conditioning. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 31:151-159.

Corbille AG, Valjent E, Marsicano G, Ledent C, Lutz B, Herve D, Girault JA (2007) Role of cannabinoid type 1 receptors in locomotor activity and striatal signaling in response to psychostimulants. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:6937-6947.

Con formato: Inglés (Estados Unidos)

De Camilli P, Harris SM, Jr., Huttner WB, Greengard P (1983) Synapsin I (Protein I), a nerve terminal-specific phosphoprotein. II. Its specific association with synaptic vesicles demonstrated by immunocytochemistry in agarose-embedded synaptosomes. The Journal of cell biology 96:1355-1373.

Dias C, Lachize S, Boilet V, Huitelec E, Cador M (2004) Differential effects of dopaminergic agents on locomotor sensitisation and on the reinstatement of cocaine-seeking and food-seeking behaviour. Psychopharmacology 175:414-427.

Ehrlich ME, Sommer J, Canas E, Unterwald EM (2002) Periadolescent mice show enhanced DeltaFosB upregulation in response to cocaine and ~~amph~~amphetamine~~etamine~~. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:9155-9159.

Ferguson SM, Thomas MJ, Robinson TE (2004) Morphine-induced c-fos mRNA expression in striatofugal circuits: modulation by dose, environmental context, and drug history. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 29:1664-1674.

Harada T, Morooka T, Ogawa S, Nishida E (2001) ERK induces p35, a neuron-specific activator of Cdk5, through induction of Egr1. Nature cell biology 3:453-459.

Hawasli AH, Benavides DR, Nguyen C, Kansy JW, Hayashi K, Chambon P, Greengard P, Powell CM, Cooper DC, Bibb JA (2007) Cyclin-dependent kinase 5 governs learning and synaptic plasticity via control of NMDAR degradation. Nature neuroscience 10:880-886.

Humbert S, Dhavan R, Tsai L (2000) p39 activates cdk5 in neurons, and is associated with the actin cytoskeleton. Journal of cell science 113 (Pt 6):975-983.

Con formato: Inglés (Estados Unidos)

Ikiz B, Przedborski S (2008) A sequel to the tale of p25/Cdk5 in neurodegeneration. Neuron 60:731-732.

Jackson HC, Nutt DJ (1993) A single preexposure produces sensitization to the locomotor effects of cocaine in mice. Pharmacology, biochemistry, and behavior 45:733-735.

Jeong J, Park YU, Kim DK, Lee S, Kwak Y, Lee SA, Lee H, Suh YH, Gho YS, Hwang D, Park SK (2013) Cdk5 phosphorylates dopamine D2 receptor and attenuates downstream signaling. PloS one 8:e84482.

Kameda SR, Fukushiro DF, Trombin TF, Procopio-Souza R, Patti CL, Hollais AW, Calzavara MB, Abilio VC, Ribeiro RA, Tufik S, D'Almeida V, Frussa-Filho R (2011) Adolescent mice are more vulnerable than adults to single injection-induced behavioral sensitization to ~~amph~~amphetamine~~etamine~~. Pharmacology, biochemistry, and behavior 98:320-324.

Kelz MB, Chen J, Carlezon WA, Jr., Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ, Marotti L, Self DW, Tkatch T, Baranauskas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR, Nestler EJ (1999) Expression of the transcription factor deltaFosB in the brain controls sensitivity to cocaine. Nature 401:272-276.

Krapacher FA, Mlewski EC, Ferreras S, Pisano V, Paolorossi M, Hansen C, Paglini G (2010) Mice lacking p35 display hyperactivity and paradoxical response to psychostimulants. Journal of neurochemistry 114:203-214.

Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, Neve RL, Self DW, Nestler EJ (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303-314.

Con formato: Inglés (Estados Unidos)

Lai KO, Ip NY (2009) Recent advances in understanding the roles of Cdk5 in synaptic plasticity. Biochimica et biophysica acta 1792:741-745.

McClung CA, Nestler EJ (2003) Regulation of gene expression and cocaine reward by CREB and DeltaFosB. Nature neuroscience 6:1208-1215.

Mlewski EC, Krapacher FA, Ferreras S, Paglini G (2008) Transient enhanced expression of Cdk5 activator p25 after acute and chronic d-~~amph~~amphetamine~~etamine~~ administration. Annals of the New York Academy of Sciences 1139:89-102.

Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nature reviews Neuroscience 2:119-128.

Nestler EJ, (2013) Cellular basis of memory for addiction. Dialogues Clin Neurosci, 15(4), 431-443.

Paglini G, Caceres A (2001) The role of the Cdk5--p35 kinase in neuronal development. European journal of biochemistry / FEBS 268:1528-1533.

Paz MC, Marchese NA, Cancela LM, Bregonzio C (2013) Angiotensin II AT(1) receptors are involved in neuronal activation induced by ~~amph~~amphetamine~~etamine~~ in a two-injection protocol. BioMed research international 2013:534817.

Pigino G, Paglini G, Ulloa L, Avila J, Caceres A (1997) Analysis of the expression, distribution and function of cyclin dependent kinase 5 (cdk5) in developing cerebellar macroneurons. Journal of cell science 110 (Pt 2):257-270.

Robinson TE, Becker JB, Presty SK (1982) Long-term facilitation of ~~amph~~amphetamine~~etamine~~-induced rotational behavior and striatal dopamine release produced by a single exposure to ~~amph~~amphetamine~~etamine~~: sex differences. Brain Res 253:231-241.

Con formato: Inglés (Estados Unidos)

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain research Brain research reviews 18:247-291.

Robinson TE, Berridge KC (2003) Addiction. Annual review of psychology 54:25-53.

Robison AJ, Vialou V, Mazei-Robison M, Feng J, Kourrich S, Collins M, Wee S, Koob G, Turecki G, Neve R, Thomas M, Nestler EJ (2013) Behavioral and structural responses to chronic cocaine require a feedforward loop involving DeltaFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:4295-4307.

Singer BF, Forneris J, Vezina, P (2014) Inhibiting cyclin-dependent kinase 5 in the nucleus accumbens enhances the expression of amphetamine-induced locomotor conditioning. Behav Brain Res, 275, 96-100. (a).

Singer BF, Neugebauer NM, Forneris J, Rodvelt KR, Li D, Bubula N, Vezina P (2014) Locomotor conditioning by amphetamine requires cyclin-dependent kinase 5 signaling in the nucleus accumbens. Neuropharmacology, 85, 243-252. (b).

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev, 24(4), 417-463.

Stewart J, Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. Behavioural pharmacology 4:289-312.

Stipanovich A, Valjent E, Matamales M, Nishi A, Ahn JH, Maroteaux M, Bertran-Gonzalez J, Brami-Cherrier K, Enslen H, Corbille AG, Filhol O, Nairn AC, Greengard P, Herve D,

Con formato: Inglés (Estados Unidos)

Girault JA (2008) A phosphatase cascade by which rewarding stimuli control nucleosomal response. Nature 453:879-884.

Takahashi S, Ohshima T, Cho A, Sreenath T, Iadarola MJ, Pant HC, Kim Y, Nairn AC, Brady RO, Greengard P, Kulkarni AB (2005) Increased activity of cyclin-dependent kinase 5 leads to attenuation of cocaine-mediated dopamine signaling. Proceedings of the National Academy of Sciences of the United States of America 102:1737-1742.

Tang D, Yeung J, Lee KY, Matsushita M, Matsui H, Tomizawa K, Hatase O, Wang JH (1995) An isoform of the neuronal cyclin-dependent kinase 5 (Cdk5) activator. The Journal of biological chemistry 270:26897-26903.

Taylor JR, Lynch WJ, Sanchez H, Olausson P, Nestler EJ, Bibb JA (2007) Inhibition of Cdk5 in the nucleus accumbens enhances the locomotor-activating and incentive-motivational effects of cocaine. Proc Natl Acad Sci U S A, 104(10), 4147-4152.

Tsai LH, Delalle I, Caviness VS, Jr., Chae T, Harlow E (1994) p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. Nature 371:419-423.

Valjent E, Bertran-Gonzalez J, Aubier B, Greengard P, Herve D, Girault JA (2010) Mechanisms of locomotor sensitization to drugs of abuse in a two-injection protocol. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 35:401-415.

Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, Greengard P, Herve D, Girault JA (2005) Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in

Con formato: Inglés (Estados Unidos)

the striatum. Proceedings of the National Academy of Sciences of the United States of America 102:491-496.

Vanderschuren LJ, Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 151:99-120.

Vanderschuren LJ, Schmidt ED, De Vries TJ, Van Moorsel CA, Tilders FJ, Schoffelmeer AN (1999) A single exposure to ~~amph~~amphetamine~~etamine~~ is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:9579-9586.

Vezina P, Leyton M (2009) Conditioned cues and the expression of stimulant sensitization in animals and humans. Neuropharmacology 56 Suppl 1:160-168.

Wolf ME, Xue CJ (1998) ~~Amph~~Amphetamine~~etamine~~ and D1 dopamine receptor agonists produce biphasic effects on glutamate efflux in rat ventral tegmental area: modification by repeated ~~amph~~amphetamine~~etamine~~ administration. Journal of neurochemistry 70:198-209.

Con formato: Inglés (Estados Unidos)

Con formato: Inglés (Estados Unidos)

Figure legends

Figure 1: Total counts of locomotor activity were registered for one hour during Training (first injection, 4mg/kg of ~~amph~~amphetamine) and Challenge (second injection, 2mg/kg ~~amph~~amphetamine). On both days, the animals were evaluated in the HOME condition (A) and in the NOVEL condition (B). Error bars represent standard error of the mean (SEM). For A: *Differences between ~~amph~~amph/sal and ~~amph~~amph/~~amph~~amph vs sal/sal and sal/~~amph~~amph groups. $^{\&}$Differences between sal/~~amph~~amph and ~~amph~~amph/~~amph~~amph vs sal/sal and ~~amph~~amph/sal groups. For B: *Differences between ~~amph~~amph/sal and ~~amph~~amph/~~amph~~amph vs sal/sal and sal/~~amph~~amph groups. $^{\&}$Differences between sal/~~amph~~amph and ~~amph~~amph/~~amph~~amph vs sal/sal and ~~amph~~amph/sal groups. $^{\#}$Differences between ~~amph~~amph/~~amph~~amph and sal/~~amph~~amph groups. $^{*,\&,\#}p < 0.05$. (amph: amphetamine)

Figure 2: Representative western immunoblots showing the levels of expression of p35 protein in SFs of the dorsal striatum of animals from the HOME condition, sacrificed 1 h (A) or 4 h (B) after Challenge. At any of the times tested, an ANOVA did not detect any differences in p35 levels between groups. Error bars represent standard error of the mean (SEM).

Con formato: Inglés (Estados Unidos)

Figure 3

Expression levels of p35 protein in SFs of the dorsal striatum of animals from the NOVEL condition, sacrificed 1 h (A) or 4 h (B) after Challenge. Note the increase in p35 levels in the ~~amph~~amph/~~amph~~amph group compared with the remaining groups (~~amph~~amph/sal or sal/~~amph~~amph) at both time points. *In vitro* Cdk5 activity of SFs of the dorsal striatum of animals from the NOVEL condition, sacrificed 1 h (C) or 4 h (D) after Challenge. Cdk5 was immunoprecipitated from individual fractions and subjected to kinase assay using histone H1 as the substrate. Note that Cdk5 activity increased dramatically in SFs of sensitized rats (group ~~amph~~amph/~~amph~~amph) at both testing times. *Differences between ~~amph~~amph/~~amph~~amph group and their control groups. Error bars represent standard error of the mean (SEM). *$p < 0.05$. (amph: amphetamine)