HHS Public Access

Author manuscript

Neuroscience. Author manuscript; available in PMC 2018 March 13.

Published in final edited form as:

Neuroscience. 2015 January 29; 285: 34–46. doi:10.1016/j.neuroscience.2014.11.013.

SELF ADMINISTRATION OF OXYCODONE ALTERS SYNAPTIC PLASTICITY GENE EXPRESSION IN THE HIPPOCAMPUS DIFFERENTIALLY IN MALE ADOLESCENT AND ADULT MICE

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Abstract

Abuse and addiction to prescription opioids such as oxycodone (a short-acting Mu opioid receptor (MOP-r) agonist) in adolescence is a pressing public health issue. We have previously shown differences in oxycodone self-administration behaviors between adolescent and adult C57BL/6J mice and expression of striatal neurotransmitter receptor genes, in areas involved in reward. In this study, we aimed to determine whether oxycodone self-administration differentially affects genes regulating synaptic plasticity in the hippocampus of adolescent compared to adult mice, since the hippocampus may be involved in learning aspects associated with chronic drug self administration. Hippocampus was isolated for mRNA analysis from mice that had self administered oxycodone (0.25 mg/kg/infusion) 2 h/day for 14 consecutive days or from yoked saline controls. Gene expression was analyzed with real-time polymerase chain reaction (PCR) using a commercially available “synaptic plasticity” PCR array containing 84 genes. We found that adolescent and adult control mice significantly differed in the expression of several genes in the absence of oxycodone exposure, including those coding for mitogen-activated protein kinase, calcium/calmodulin-dependent protein kinase II gamma subunit, glutamate receptor, ionotropic AMPA2 and metabotropic 5. Chronic oxycodone self administration increased proviral integration site 1 (*Pim1*) and thymoma viral proto-oncogene 1 mRNA levels compared to controls in both age groups. Both *Pim1* and cadherin 2 mRNAs showed a significant combined effect of Drug Condition and Age × Drug Condition. Furthermore, the mRNA levels of both cadherin 2 and cAMP response element modulators showed an experiment-wise significant difference between oxycodone and saline control in adult but not in adolescent mice. Overall, this study demonstrates for the first time that chronic oxycodone self-administration differentially alters synaptic plasticity gene expression in the hippocampus of adolescent and adult mice.

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Keywords

oxycodone self-administration; hippocampus; synaptic plasticity; gene expression; adult; adolescent

INTRODUCTION

Prescription opioid abuse poses a significant public health issue in the United States (Zosel et al., 2013). This problem has escalated over the past two decades, especially among adolescents (Compton and Volkow, 2006; Johnston et al., 2006; Johnston, 2009), of whom thousands are hospitalized every year as a result of non-medical use of prescription opioids (Zosel et al., 2013). This issue is particularly troublesome because little is known about how the rapidly changing adolescent brain is affected by exposure to prescription opioids (Compton and Volkow, 2006). As a result of the high degree of neuroplasticity during adolescence (Carpenter-Hyland and Chandler, 2007), the neurobiological alterations that adolescents experience in response to prescription opioids may be mechanistically different, or may persist into adulthood, thus conferring on them a greater vulnerability to opioid addiction upon subsequent abuse.

As drugs of abuse have been shown to engage the molecular mechanisms of learning and memory by affecting synaptic plasticity (Berke and Hyman, 2000; Kauer and Malenka, 2007), understanding the effects of addictive drugs on brain regions involved in learning and memory, such as the hippocampus (Billa et al., 2010) is crucial. The hippocampus has been shown to be integral to the storage, consolidation, and retrieval of declarative, spatial, and associative long-term memory (Hernandez-Rabaza et al., 2007), with long-term potentiation (LTP) and long-term depression (LTD) as representative measures of these processes (Caruana et al., 2012). Due to its role in learning and memory and through its efferent and afferent neural connections with the reward system (Kenney and Gould, 2008; Garcia-Fuster et al., 2011), the hippocampus is important to the response to addictive drugs. In particular, the hippocampus has been shown to be involved in context-drug associations (Kenney and Gould, 2008), reward-related response (Bao et al., 2007; Hernandez-Rabaza et al., 2007), as well as drug craving (Volkow, 2004) and seeking behavior (Vorel et al., 2001; Belujon and Grace, 2011), especially in the context of reinstatement and relapse to drugs of abuse (Robbins and Everitt, 2002; Hernandez-Rabaza et al., 2007; Belujon and Grace, 2011). Furthermore, due to its role in modulating the hypothalamic–pituitary-adrenal (HPA) axis, the hippocampus plays a key role in stress-induced drug-seeking behavior (Garcia-Fuster et al., 2011).

There have been studies showing that opiates, morphine in particular, alter gene expression in several brain regions in adult rodents (Wang et al., 1999; Nestler, 2001; Rodriguez Parkitna et al., 2004; Korostynski et al., 2006; Hassan et al., 2010). One such study, which involved morphine-induced conditioned place preference, showed changes in the expression of genes in the hippocampus involved in vesicular transport, neurotransmitter release, and receptor trafficking (Marie-Claire et al., 2007). However, there are few studies detailing the effects of prescription opioids on changes in gene expression in the adolescent brain (Ellgren

et al., 2007). Furthermore, much of the research on the effects of drugs of abuse on the adolescent hippocampus has focused on nicotine, alcohol, and cannabis. For example, one study demonstrated that adolescent rats given alcohol showed more hippocampal changes in protein expression relative to adolescent controls compared to adult rats given alcohol (Hargreaves et al., 2009).

We recently reported that adolescent mice self-administered significantly less oxycodone than adult mice (in the same mice described in the study presented here) (Mayer-Blackwell et al., 2014). We hypothesized that there were differential neurobiological alterations in brain regions associated with reward between adolescent and adult mice as a consequence of oxycodone self-administration. In this study, we examined this hypothesis by measuring the expression of genes involved in synaptic plasticity in the hippocampus. This study is in agreement with our previous finding that adolescent mice that self-administered oxycodone experienced different changes in gene expression of neurotransmitter receptors in the dorsal striatum relative to controls than did adult mice that self-administered oxycodone (Mayer-Blackwell et al., 2014).

EXPERIMENTAL PROCEDURES

Subjects

Male adolescent and adult (4 or 11 weeks old on arrival, respectively) C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) were housed in groups up to five with free access to food and water in a light-(12:12-h light/dark cycle, light on at 7:00 pm and off at 7:00 am) and temperature-(25 °C) controlled room. Animal care and experimental procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences 1996). The experimental protocols used were approved by the Institutional Animal Care and Use Committee of The Rockefeller University.

Self-administration procedure

Catheter implantation—Following acclimation for 7 days, the mice were anesthetized with a combination of xylazine (8.0 mg/kg i.p.) and ketamine (80 mg/kg i.p.). After shaving and application of a 70% alcohol and iodine preparatory solution, incisions were made in the midscapular region and anteromedial to the foreleg. A catheter of approximately 6 cm in length (ID: 0.31 mm, OD: 0.64 mm) (Helix Medical, Inc., Carpinteria, CA, USA) was passed subcutaneously from the dorsal to the ventral incision. A 22-gauge needle was inserted into the jugular vein to guide the catheter into the vein. The catheter was tied to the vein with surgical silk. Physiological saline was then flushed through the catheter to avoid clotting and the catheter then capped with a stopper. Antibiotic ointment was applied to the catheter exit wounds on the animal's back and forearm. Mice were individually housed after the surgery and were allowed 4 days of recovery (due to the limited period of adolescence in the mouse (Adriani and Laviola, 2004; Spear, 2000) before being placed in operant test chambers for the self-administration procedure.

Operant conditioning chambers—The self-administration chamber, ENV-307W (21.6 cm × 17.8 cm × 12.7 cm, Med. Associates, St. Albans, VT, USA), was located inside a larger sound attenuation chamber (Med. Associates). Each chamber contained a wall with two small holes (0.9-cm diameter, 4.2 cm apart, 1.5 cm from the floor of the chamber). One hole was defined as active, the other was inactive. When the photocell in the active hole was triggered by a nose-poke, an infusion pump (Med. Associates) delivered an oxycodone infusion of 20 μl/3 s from a 5-ml syringe. The syringe was connected by a swivel via Tygon tubing. The infusion pump and syringe were outside the chamber. During infusion, a cue light above the active hole was illuminated. Each injection was followed by a 20-s "time-out" period during which poking responses were recorded but had no programed consequences. All responses at the inactive hole were also recorded. Mice were tested during the dark phase of the diurnal cycle (all experiments were performed between 8:00 am and 12:00 pm).

Oxycodone self-administration—A 2-h self-administration session was conducted daily. Mice were weighed and heparinized saline solution (0.02 ml of 30 IU/ml) was used daily to flush the catheter to maintain patency. During self-administration sessions, mice in the oxycodone (Sigma, St. Louis, MO, USA) groups were placed in the self-administration chamber and a nose-poke through the active hole led to an infusion of oxycodone (0.25 mg/kg/infusion) under an FR1 schedule for 14 days. Drug volume was controlled by a computer to follow daily changes in body weight of individual animals. Mice in the control groups received yoked saline infusions during all sessions (saline was infused in the control mouse whenever the oxycodone mouse self-administered oxycodone). At the end of the experiment, only data from mice that passed a catheter patency test (defined as loss of muscle tone within a few seconds after administration of a short-acting anesthetic) with injection of 30 μl of ketamine (5 mg/ml) (Fort Dodge, IA) were included in the analysis of data. See also Mayer-Blackwell et al. (2014).

RNA extraction

Mice were sacrificed within 1 h after the last self-administration session by decapitation after brief exposure to CO_2; the whole hippocampus from each mouse was dissected from the brain and homogenized in Qiazol (Qiagen, Valencia, CA, USA). Total RNA was isolated from homogenates of the hippocampus using the miRNeasy kit (Qiagen, Valencia, CA, USA). The quality and quantity of RNA from each sample was determined using the Agilent 2100 bioanalyzer. Genomic DNA was removed from the isolated total RNA using RT2 HT First Strand Kit (Qiagen, Valencia, CA, USA). Complementary DNA was then synthesized from 500 ng of total RNA with the same kit.

Mouse synaptic plasticity RT^2-Profiler™ polymerase chain reaction (PCR) array

The mouse synaptic plasticity RT^2 Profiler™ PCR array (PAMM-126Z, Qiagen) profiles the expression of 84 genes involved in synaptic plasticity and five housekeeping genes (glyceraldehyde 3-phosphate dehydrogenase, Gapdh; hypoxanthine guanine phosphoribosyl transferase 1, Hprt-1; Heat shock protein 90 alpha (cytosolic), class B member 1, Hsp90ab1 and β-glucuronidase, Gusb; β-actin, Actb) by real-time PCR using the SYBR Green detection method. The generated cDNA was diluted with an appropriate volume of

instrument-specific reagent (2× SuperArray RT2 Real-Time™ SYBR Green PCR Master Mix (PA-012) and ultra pure water) and 10 μl of this reaction mix was added to each well of the PCR array. The real-time PCR reaction was performed in an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA), applying the following program: 2 min at 50 °C, 10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The ABI Prism 7900 HT Sequence Detection System was used to calculate the Ct value for each well. Data were normalized to the mean of the five housekeeping genes (none of which showed differences between oxycodone and yoked saline controls) and analyzed by the comparative Ct-method (2-ΔCT). The complete list of genes assayed on the array is shown in Table 6 and can also be found at the manufacturer's website. (http://www.sabioscience.com/rt_pcr_product/HTML/PAMM-126Z-4.html).

Statistical analysis

Differences in expression of genes were analyzed by a two-way analysis of variance (Age × Drug Condition). "Age" would indicate a difference of mean measurements between adolescents and adults; "Drug Condition" would indicate a difference between cases and controls; and "Age × Drug Condition" would indicate a difference in the Age effect of the two Drug Condition classes, or a difference in Drug Condition effect for the two Age classes. As the main interest is on Drug Condition and Age × Drug Condition Interaction, for each of the 84 genes, *p*-values for Drug Condition and Age × Drug Condition are combined by the Fisher method, resulting in values shown in Table 4; this value indicates a combined effect of Drug Condition and its interaction with Age, that is, a difference in Drug Condition in the two Age groups.

A correction for multiple testing is the Bonferroni method. Here, we use the number of genes, $n = 84$, as the correction factor. While three tests were carried out for each gene, the Bonferroni correction is known to be conservative and assumes independent genes. As expression levels for different genes are correlated (for all individuals, the average absolute value of the correlation coefficient over all pairs of genes is equal to 0.27), the correction factor may thus be appropriate; a corrected significance threshold is given by $0.05/84 \approx 0.0006$; any *p*-value equal to 0.0006 or smaller was therefore considered experiment-wise significant at the 5% level.

To obtain an accurate picture of p-values corrected for multiple testing, permutation analysis was carried out with a version of our *sumstat* program (http://lab.rockefeller.edu/ott/programs). In each of the two age groups, a *t*-test was carried out for each of the 84 genes. *P*-values were estimated in 100,000 randomization samples. Results are shown in Table 5. "p0Stat" is an uncorrected *p*-value while "pStat" refers to *p*-values corrected for testing 84 genes. We refer to point-wise significance as being represented by a nominal *p*-value of 0.05 or less, and experiment-wise significance as a corrected *p*-value of 0.05 or less.

RESULTS

Gene expression differing between adolescent and adult mice

A two-way ANOVA, Age × Drug Condition, found that there were thirteen genes showing a point-wise significant main effect of Age, see Table 1. Only the four genes showing experiment-wise significance are detailed below. Only the results of individual analysis of variance for each of these genes are shown in the Fig. 1.

Mitogen-activated protein kinase 1 (Mapk1)—The effect of age and oxycodone self-administration on *Mapk1* mRNA expression in the hippocampus is shown in Fig. 1A. A two-way ANOVA showed a significant main effect of Age, $F(1, 19) = 22.80$, $p < 0.0002$, and Drug Condition, $F(1, 19) = 8.34$, $p < 0.01$, without significant interaction. There were lower *Mapk1* mRNA levels in adolescent mice than in the adult mice. The mRNA levels of *Mapk1* were higher in mice that had self-administered oxycodone compared with those of the yoked saline controls.

Calcium/calmodulin-dependent protein kinase II gamma (Camk2g)—The effect of age on *Camk2g* mRNA expression in the hippocampus is shown in Fig. 1B. A two-way ANOVA showed that there was a significant main effect of Age, $F(1, 19) = 20.02$, $p < 0.0005$. There were significantly lower levels of *Camk2g* mRNA in the adolescent mice than in the adult mice.

Glutamate receptor, ionotropic AMPA2 (Gria2)—The effect of age on *Gria2* mRNA expression in the hippocampus is shown in Fig. 1C. A two-way ANOVA showed that there was a significant main effect of Age, $F(1, 19) = 20.06$, $p < 0.0005$. The levels of *Gria2* mRNA in the adolescent mice were lower than those of the adult mice.

Glutamate receptor, metabotropic 5 (Grm5)—The effect of age on *Grm5* mRNA expression in the hippocampus is shown in Fig. 1D. A two-way ANOVA showed there was a significant main effect of Age, $F(1, 19) = 17.71$, $p < 0.0005$. There were significantly lower levels of *Grm5* mRNA in adolescent mice than in adult mice.

Gene expression differing between oxycodone groups and yoked saline controls

Two-way ANOVAs showed that there were fifteen genes showing a point-wise significant main effect of Drug Condition, see Table 2. Only the two genes with experiment-wise significant differences are detailed below. Only the results of individual analysis of variance for each of these genes are shown in the Fig. 2.

Proviral integration site 1 (Pim1)—The effect of oxycodone self-administration on *Pim1* mRNA levels in the hippocampus is shown in Fig. 2A. A two-way ANOVA showed a significant main effect of Drug Condition, $F(1, 19) = 22.65$, $p = 0.0001$. There were higher levels of *Pim1* mRNA in the hippocampus of mice that self-administered oxycodone compared to those of yoked-saline controls.

Thymoma viral proto-oncogene 1 (Akt1)—The effect of oxycodone self-administration on *Akt1* mRNA levels in the hippocampus is shown in Fig. 2B. A two-way

ANOVA revealed a significant main effect of Drug Condition, $F(1, 19) = 38.98$, $p = 0.0005$. Mice that had self-administered oxycodone had higher levels of *Akt1* mRNA than those of the yoked-saline controls.

Gene expression showing a significant of Age × Drug Condition Interaction

Two-way ANOVAs showed that there were six genes showing a point-wise significant Age × Drug Condition Interaction, with no genes showing experiment-wise significance, see Table 3.

Gene expression showing a significant combined effect of Drug Condition and Age × Drug Condition Interaction

Two-way ANOVAs showed that there were twenty-two genes showing a point-wise significant combined effect of Drug Condition and Age × Drug Condition Interaction, see Table 4. Only the two genes with experiment-wise significant differences are detailed below.

Cadherin 2 (Cdh2)—The effect of oxycodone self-administration on *Cdh2* mRNA levels in the hippocampus following 14-day oxycodone self-administration or yoked saline in adolescent and adult mice is shown in Fig. 2C. Two-way ANOVAs, Age × Drug Condition, showed a significant main effect of Age, $F(1, 19) = 6.21$, $p < 0.05$, a significant main effect of Drug Condition, $F(1, 19) = 15.74$, $p < 0.001$, and a significant Age × Drug Condition Interaction, $F(1, 19) = 9.72$, $p < 0.01$. The Fisher method showed that there was a significant combined Drug Condition and Age × Drug Condition effect, $p < 0.0001$. There were higher levels of *Cdh2* mRNA in the hippocampus of mice that self-administered oxycodone compared to those of yoked-saline controls. The interaction effect was seen as an increase in mean value from adolescent to adult for oxycodone self-administering mice but a decrease in mean value from adolescent to adult in yoked saline controls.

Proviral integration site 1 (Pim1)—The effect of oxycodone self-administration on *Pim1* mRNA levels is presented in Fig. 2A. The Fisher method showed that there was a significant combined main effect of Drug Condition and Age × Drug Condition Interaction, $p < 0.001$. Self administration led to higher levels of *Pim1* mRNA in the hippocampus of adolescent and adult mice compared to those of yoked-saline controls.

Significant differences in gene expression by permutation analysis

Permutation analysis showed only two genes with experiment-wise significance between the oxycodone and saline controls in the adult mice, whereas none of the genes examined from adolescent mice showed experiment-wise significant difference in expression (see Table 5). In the adult groups, *Cdh2* mRNA levels were significantly higher in the oxycodone self-administering group than in saline controls, $p < 0.05$ (Fig. 3A). In addition, cAMP responsive element modulator (*Crem*) mRNA levels were significantly higher in the oxycodone self-administering mice than in yoked saline control, $p < 0.05$ (Fig. 3B).

Correlation between the amount of oxycodone self administered and Crem mRNA levels

To quantify the relationship between the amounts of oxycodone self administered and alteration in mRNA levels following 14-days of oxycodone self administration, we

examined the correlation between the levels of mRNA of each gene showing changes in response to oxycodone self administration and the amount of oxycodone self administered across all sessions or during the last session by each mouse. We found that there was a significant positive correlation between the amounts of oxycodone self-administered during the last self administration session and *Crem* mRNA levels, $r = 0.960$, $p < 0.01$ in the adult mice (Fig. 4A). There was also a significant positive correlation between the total amounts of oxycodone self-administered and *Crem* mRNA levels, $r = 0.8985$, $p < 0.05$ across the adult mice examined (Fig. 4B). No significant correlations were found in any other genes examined in this study (data not shown).

DISCUSSION

In the current study, we found that oxycodone self-administration significantly altered a number of genes involved in synaptic plasticity in the hippocampus of both the adolescent and adult mice. These genes included proviral integration site 1 and thymoma viral proto-oncogene 1, which showed experiment-wise significance between the oxycodone and saline groups. Proviral integration site 1 kinase is a short-lived serine/threonine kinase. *Pim1* belongs to Ca2+/Calmodulin-dependent protein kinase family. *Pim* kinases are highly expressed in various tumors and enhance cell proliferation, survival (Muraski et al., 2007) and attenuate apoptosis (Shirogane et al., 1999). Thymoma viral proto-oncogene 1 is also a serine-threonine protein kinase. *Akt* is a critical mediator of growth factor-induced neuronal survival. The *Akt* pathway plays a critical role in mediating signal transduction in cell proliferation, differentiation and survival (e.g., Shao et al., 2010; Wang et al., 2012).

Mu opioid receptor (MOP-r) agonists such as morphine and heroin have been shown to cause structural plasticity in the hippocampus. Decrease in neurogenesis by 42% in the adult rat hippocampal granule cell layer was found following chronic administration of the MOP-r agonist morphine. A similar effect was also found in rats after chronic self-administration of heroin (Eisch et al., 2000). In a separate study, repeated morphine treatment led to a significant reduction of cellular proliferation in morphine-dependent animals, which rebounded after 1-week withdrawal and returned to normal after 2-week withdrawal (Kahn et al., 2005). Further, it was found that chronic morphine administration decreased neurogenesis by inhibiting dividing cells and progenitor cell progression to a more mature neuronal stage in the adult hippocampal subgranular zone (Arguello et al., 2008). Similar to the action of morphine, oxycodone is a short acting MOP-r agonist. Although there have been no reports of inhibition of neurogenesis by oxycodone, we speculate that oxycodone self-administration could also lead to decreased neurogenesis in the hippocampus. Thus, increases in both *Pim1* and *Akt1* kinases found in the mice that had self administered oxycodone for 14 days may be a compensatory response to inhibition of neurogenesis in the hippocampus induced by long-term oxycodone exposure.

MOP-r agonists have also been shown to modulate hippocampal functional plasticity. One study found that morphine dependence (i.e. after chronic morphine administration) attenuated the induction of LTP in rat hippocampal slices (Salmanzadeh et al., 2003). Another study demonstrated that while chronic morphine and heroin treatment reduced LTP in the rat hippocampus during withdrawal, this reduction in LTP could be reversed by re-

exposure to the opiates (Pu et al., 2002). However, in a more recent study, heroin and morphine were shown to differentially alter hippocampal LTP since re-exposure to morphine restored reduced LTP during withdrawal in heroin-dependent rats, while heroin could not restore reduced LTP during withdrawal in morphine-dependent rats (Bao et al., 2007). This study demonstrates the importance of conducting experiments using other specific opiates, such as oxycodone, as they may have diverging effects on synaptic plasticity.

Mitogen-activated protein kinase and calcium/calmodulin-dependent protein kinase II are critical for synaptic plasticity and memory formation. One study found that levels of Mapk, CamkII a and Akt increased 24 h after inhibitory avoidance training in the rat hippocampus (Bekinschtein et al., 2010). Another study showed that phosphorylation levels of Mapk1/2, alpha subunit of CaMKII and Akt rapidly increased during early LTP at Schaffer collateral-CA1 mouse hippocampal synapses (Racaniello et al., 2009). Such increases in proteins kinases suggest that these kinases are closely involved in memory consolidation. In the present study, the expression of a number of genes was found to differ between the adult and adolescent hippocampus in the absence of oxycodone exposure, among them, *Mapk1*, *CamkII g*, *Gria2* and *Grm5* show experiment-wise significant differences. The significantly higher levels of expressions, especially in *Mapk1* and *CamkIIg*, in adult hippocampus compared to adolescent hippocampus are important. Anatomically, neurocircuitries involved in learning and memory may not be as well developed within the adolescent hippocampus as in the adult. In addition, *Mapk1* and *CamkIIg*, key elements for regulating synaptic plasticity and memory consolidation, are significantly lower in the adolescent hippocampus compared to those of the adult. These may, in part, determine more solid memory in adults than in adolescents.

Cadherin 2 (*Cdh2*) is a calcium-dependent adhesion transmembrane protein. The cadherin superfamily plays important roles in cell adhesion and forms adherent junctions to bind cells within tissues together. N-cadherin is abundantly expressed in migrating cells in the subventricular zone of the hippocampus and plays important roles in forming cell clusters and in regulating cell differentiation in this region (Yagita et al., 2009). One study found that persistence of coordinated LTP and dendritic spine enlargement at mature hippocampal CA1 synapses requires N-cadherin (Bozdagi et al., 2010). In another study, it was found that hippocampal N-cadherin mediated memory consolidation, at least in part, via cytoskeletal *Mapk* signaling (Schrick et al., 2007). The current study found that adult mice that self administered oxycodone had significantly higher *Cdh2* mRNA levels compared to yoked saline control; which was not found in adolescent mice. The increases in *Cdh2* gene expression may be closely associated with oxycodone self administration-related memory in the adult hippocampus. The lack of response of *Cdh2* mRNA found in the adolescent hippocampus might be related to lower intake of oxycodone by adolescent compared with the adult mice (Mayer-Blackwell et al., 2014).

Crem encodes cAMP-responsive element modulator, binding to the cAMP responsive element, regulating transcription factors. This regulation is an important mechanism mediating the brain's adaptation to changing environments. One study found that intracranial self stimulation upregulated the expression of synaptic plasticity-related genes including *Crem* in the rat hippocampus (Kadar et al., 2013). Other studies showed that the

inducible cAMP early repressor (icer), encoded by *Crem*, plays an important role in the regulation of long-term plasticity underlying learning and memory (for review, see Borlikova and Endo, 2009). Our finding that *Crem* mRNA levels significantly differed between oxycodone and saline controls only in adult but not in adolescent mice suggests that *Crem* may play an important role in the higher intake of oxycodone found in the adult mice (Mayer-Blackwell et al., 2014). This is supported by the positive correlation between the amount of oxycodone self administered and levels of *Crem* mRNA found in the adult mice, but not in the adolescent mice.

Both striatum and hippocampus are brain regions playing crucial and complementary roles in learning. Striatum (especially dorsal) is known to regulate reward and habitual learning (e.g., Ito et al., 2002; Porrino et al., 2007; Belin and Everitt, 2008) whereas the hippocampus may be related to drug self-administration associated learning and memory (including contextual learning). The positive correlation between the amount of oxycodone self administered and striatal *Maoa* mRNA levels found in our earlier study in the same mice (Mayer-Blackwell et al., 2014) suggests that oxycodone self administration induced increase in striatal *Maoa* mRNA levels may be mechanistically related to increases in striatal dopamine levels as a result of oxycodone exposure (Zhang et al., 2009). It is interesting that more neurotransmitter genes showed significant changes in the striatum in adolescents compared to adults, but fewer hippocampal synaptic plasticity genes were altered in response to oxycodone self administration. Such a finding may suggest that adolescent synaptic plasticity genes in the hippocampus are either less responsive to oxycodone self administration or that adolescent hippocampus needs to undergo maturation in order to respond to oxycodone self administration.

One *caveat* in the present study is that our main control groups (yoked saline) do not directly assess whether the observed effects are due simply to oxycodone exposure, learning components of the acquisition/performance of the behavioral assay, or a combination of both. Thus it cannot be presently determined if the observed effects, and their differences in adolescents and adults, are due to specific interactions between oxycodone exposure and hippocampal-mediated learning components.

CONCLUSION

The current study led to the first identification of differences in gene expression in the hippocampus in adolescent versus adult mice as a result of chronic self-administration of oxycodone. Our results demonstrate different transcription profiles of specific targets involved in neuroplasticity (such as, *Cdh2* and *Crem*), between adolescents and adults, providing initial avenues for future system-wide functional and mechanistic analyses.

Acknowledgments

This work was supported by NIH 1R01DA029147 (YZ) and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (MJK).

We thank Drs. Orna Levran, Vadim Yuferov, Eduardo Butelman, Molly Deutsch-Feldman, Matthew Randesi for their help in preparing the manuscript.

Abbreviations

LTP	long-term potentiation
MOP-r	Mu opioid receptor
PCR	polymerase chain reaction

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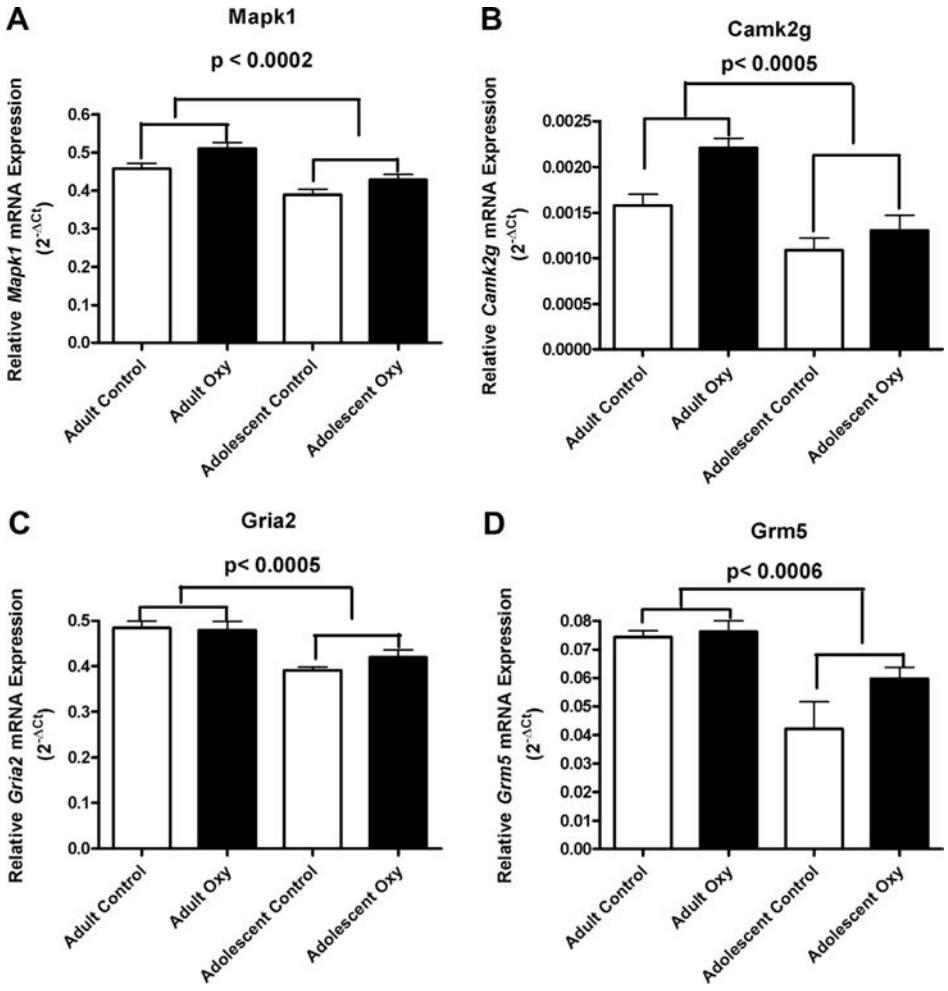

Fig. 1.

Effect of Age on *Mapk1* (A), *Camk2g* (B), *Gria 2* (C) and *Grm5* (D) mRNA expression in the hippocampus of mice that had self administrated oxycodone and yoked controls. There were significantly lower *Mapk1* mRNA levels in adolescent mice than in the adult mice, $p < 0.0002$. Also, the mRNA levels of *Mapk1* were higher in mice that had self-administered oxycodone compared with those of the yoked saline controls (1A) (± SEM), $p < 0.01$. There were significantly lower levels of *Camk2g* mRNA levels in the adolescent mice than in the adult mice ($p < 0.0005$) (1B) (±SEM). The levels of *Gria2* ($p < 0.0005$) and *Grm5* ($p < 0.0006$) mRNA in the adolescent mice were significantly lower than those of the adult mice (1C and 1D, respectively) (± SEM).

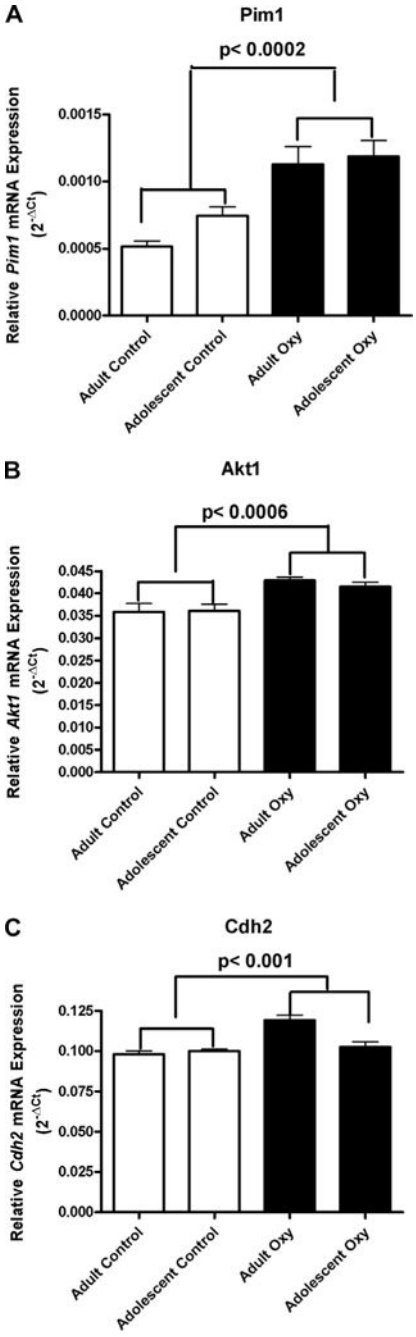

Fig. 2.

Effect of oxycodone self administration on *Pim1* (A), *Akt1* (B) and *Cdh2* (C) mRNA expression in the hippocampus. Oxycodone self administration significantly affected *Pim1* in the mouse hippocampus. *Pim1* mRNA levels increased in mice that had self administered oxycondone for 14 consecutive days in both adolescent and adult groups (2A) (± SEM). Oxycodone self administration also affected *Akt1* gene expression. Hippocampal *Akt1* mRNA levels significantly increased in both adolescent and adult mice that had self administered oxycodone compared to yoked saline controls (2B) (± SEM). The effect of oxycodone self-administration on *Cdh2* mRNA levels in the hippocampus is shown in Fig.

2C (± SEM). There were higher levels of *Cdh2* mRNA in the hippocampus of mice that self-administered oxycodone compared to those of yoked-saline controls. The interaction effect was seen as an increase in mean value from adolescent to adult for oxycodone self administered mice but a decrease in mean value from adolescent to adult in yoked saline controls.

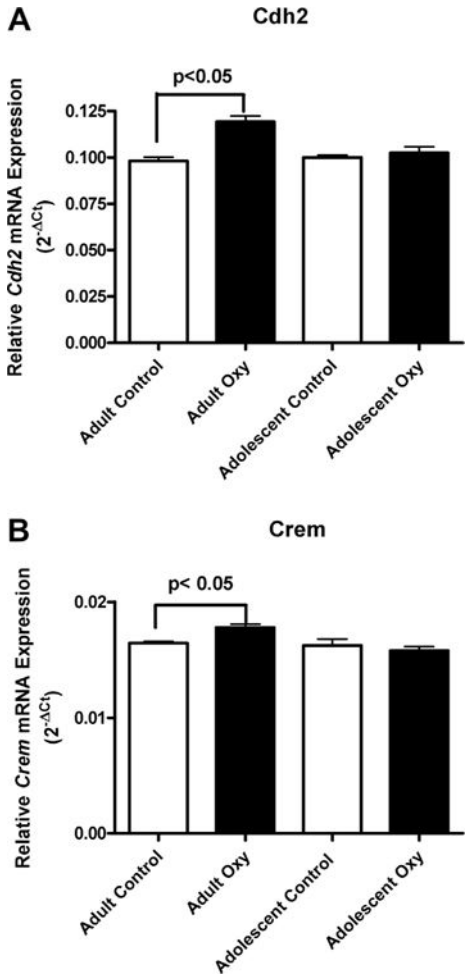

Fig. 3.

Effects of oxycodone on gene expression in the adult and adolescent mice are shown in Figure 3. In the adult groups, *Cdh2* mRNA levels were significantly higher in oxycodone self administered group than saline controls, $p < 0.05$ (Fig. 3A) (± SEM). *Crem* mRNA levels were also significantly higher in the oxycodone self administered mice than in yoked saline control, $p < 0.05$ (Fig. 3B) (± SEM).

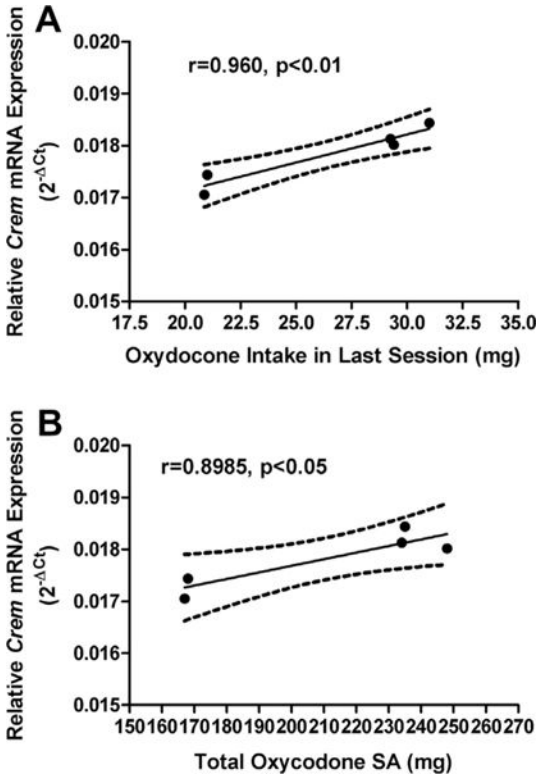

Fig. 4.

Correlation between the amount of oxycodone self administered and *Crem* mRNA levels. There was a significant positive correlation between *Crem* mRNA levels and the amounts of oxycodone self-administered in the last oxycodone self-administration session (A). There was also a significant positive correlation between *Crem* mRNA levels and the total amounts of oxycodone self-administered (B).

Table 1

Genes showing a significant main effect of Age

Gene symbol	Protein	*P* value	mRNA expression levels in adults (A) vs. adolescents (a)
Mapk1	Mitogen-activated protein kinase 1	0.0001	A > a
Camk2g	Calcium/calmodulin-dependent protein kinase II gamma	0.0003	A > a
Gria2	Glutamate receptor, ionotropic, AMPA2 (alpha 2)	0.0003	A > a
Grm5	Glutamate receptor, metabotropic 5	0.0005	A > a
Gria3	Glutamate receptor, ionotropic, AMPA3 (alpha 3)	0.0011	A > a
Grin2d	Glutamate receptor, ionotropic, NMDA2D (epsilon 4)	0.0016	A < a
Prkg1	Protein kinase, cGMP-dependent, type I	0.0025	A > a
Adcy1	Adenylate cyclase 1	0.0044	A > a
Adam10	A disintegrin and metallopeptidase domain 10	0.0044	A > a
Adcy8	Adenylate cyclase 8	0.0049	A > a
Crem	CAMP responsive element modulator	0.0055	A > a
Gnai1	Guanine nucleotide binding protein (G protein), alpha inhibiting 1	0.007	A > a
Camk2a	Calcium/calmodulin-dependent protein kinase II alpha	0.0071	A > a

Table 2

Genes showing a significant main effect of Drug Condition

Gene symbol	Protein	*P* value	Direction of change
Pim1	Proviral integration site 1	0.0001	↑
Akt1	Thymoma viral proto-oncogene 1	0.0005	↑
Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	0.0008	↑
Cdh2	Cadherin 2	0.0008	↑
Jun	Jun oncogene	0.0014	↑
Arc	Activity-regulated cytoskeletal-associated protein	0.002	↑
Junb	Jun-B oncogene	0.0032	↑
Adcy1	Adenylate cyclase 1	0.0036	↑
Nos1	Nitric oxide synthase 1, neuronal	0.0036	↑
Plcg1	Phospholipase C, gamma 1	0.0048	↑
Timp1	Tissue inhibitor of metalloproteinase 1	0.0054	↑
Tnf	Tumor necrosis factor	0.0065	↑
Mapk1	Mitogen-activated protein kinase 1	0.0094	↑
Ppp1r14a	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	0.0095	↓
Ntf5	Neurotrophin 5	0.0097	↓

Table 3

Gene expression showing a significant effect of Age × Drug Condition Interaction

Gene symbol	Protein	*P* value
Ppp1ca	Protein phosphatase 1, catalytic subunit, alpha isoform	0.0048
Cdh2	Cadherin 2	0.0057
Rgs2	Regulator of G-protein signaling 2	0.0170
Crem	cAMP-responsive element modulator	0.0211
Ppp2ca	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	0.0255
Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	0.0456

Table 4

Genes showing significant combined effect of Drug Condition and Age × Drug Condition Interaction

Gene symbol	Protein	*P* value
Cdh2	Cadherin 2	0.0001
Pim1	Proviral integration site 1	0.0005
Akt1	Thymoma viral proto-oncogene 1	0.0028
Nos1	Nitric oxide synthase 1, neuronal	0.0033
Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	0.005
Sirt1	Sirtuin 1 (silent mating type information regulation 2, homolog) 1 (S. cerevisiae)	0.0075
Tnf	Tumor necrosis factor	0.0078
Jun	Jun oncogene	0.0088
Arc	Activity regulated cytoskeletal-associated protein	0.0113
Timp1	Tissue inhibitor of metalloproteinase 1	0.0116
Ppp1r14a	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	0.0119
Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	0.0128
Junb	Jun-B oncogene	0.0177
Ntf5	Neurotrophin 5	0.0194
Ppp1ca	Protein phosphatase 1, catalytic subunit, alpha isoform	0.021
Plcg1	Phospholipase C, gamma 1	0.0225
Adcy1	Adenylate cyclase 1	0.0231
Srf	Serum response factor	0.0283
Crem	CAMP responsive element modulator	0.0285
Reln	Reelin	0.03
Mapk1	Mitogen-activated protein kinase 1	0.0397
Gria1	Glutamate receptor, ionotropic, AMPA1 (alpha 1)	0.044

Table 5

Gene expression by permutation analysis

Gene	Adolescents			Adults		
	t-stat	p0Stat	pStat	t-stat	p0Stat	pStat
Adam10	1.1919	0.2756	1.0000	1.5212	0.1642	1.0000
Adcy1	2.1002	0.0706	0.9418	2.8694	0.0262	0.6131
Adcy8	0.2943	0.7690	1.0000	0.5252	0.6176	1.0000
Akt1	2.8614	0.0198	0.5402	3.0021	0.0174	0.5310
Arc	2.6321	0.0211	0.6787	2.4483	0.0411	0.8050
Bdnf	0.2593	0.8081	1.0000	0.5910	0.5581	1.0000
Camk2a	2.1008	0.0389	0.9418	1.5416	0.1477	1.0000
Camk2g	0.8748	0.4029	1.0000	3.4958	0.0107	0.3221
Cdh2	0.5710	0.5792	1.0000	5.3723	**0.0020**	**0.0364**
Cebpb	1.8044	0.0436	0.9888	2.1524	0.0699	0.9217
Cebpd	2.6573	0.0271	0.6588	2.9977	0.0200	0.5354
Cnr1	0.5855	0.5662	1.0000	0.0409	0.9664	1.0000
Creb1	1.8359	0.0752	0.9874	1.0774	0.3104	1.0000
Crem	0.6802	0.5081	1.0000	5.1072	**0.0020**	**0.0493**
Dlg4	1.5462	0.1636	1.0000	0.6697	0.5665	1.0000
Egr1	2.0242	0.0784	0.9562	0.4780	0.7411	1.0000
Egr2	0.0608	0.9573	1.0000	0.5097	0.6190	1.0000
Egr3	0.7826	0.4496	1.0000	1.2173	0.2505	1.0000
Egr4	2.5824	0.0335	0.7109	0.8300	0.4290	1.0000
Ephb2	0.3960	0.6932	1.0000	0.9627	0.3446	1.0000
Fos	0.0579	0.9646	1.0000	1.3644	0.2089	1.0000
Gabra5	0.1930	0.8473	1.0000	0.5653	0.5786	1.0000
Gnai1	0.6283	0.5478	1.0000	0.8563	0.4024	1.0000
Gria1	2.4841	0.0338	0.7630	0.6838	0.5056	1.0000
Gria2	1.2589	0.2474	1.0000	0.1906	0.8494	1.0000
Gria3	1.2821	0.2285	1.0000	0.7410	0.4703	1.0000
Gria4	1.4551	0.1716	1.0000	0.2226	0.8182	1.0000

Gene	Adolescents			Adults		
	t-stat	p0Stat	pStat	t-stat	p0Stat	pStat
Grin1	2.1134	0.0550	0.9406	0.3648	0.7212	1.0000
Grin2a	1.2898	0.2282	1.0000	1.4780	0.1742	1.0000
Grin2b	0.7591	0.4803	1.0000	0.2403	0.8166	1.0000
Grin2c	1.3719	0.1930	1.0000	2.0639	0.0680	0.9417
Grin2d	1.7826	0.1109	0.9925	0.2956	0.7657	1.0000
Grip1	1.9917	0.0748	0.9605	0.6064	0.5936	1.0000
Grm1	0.6242	0.5464	1.0000	0.3532	0.7260	1.0000
Grm2	1.2014	0.2395	1.0000	1.1948	0.2699	1.0000
Grm3	0.0817	0.9372	1.0000	0.5415	0.6005	1.0000
Grm4	0.0409	0.9587	1.0000	0.9368	0.3795	1.0000
Grm5	1.7283	0.0834	0.9951	0.4022	0.7198	1.0000
Grm7	0.7147	0.5735	1.0000	1.6326	0.1438	0.9960
Grm8	1.0299	0.3109	1.0000	0.1287	0.8979	1.0000
Homer1	1.9550	0.0802	0.9710	0.5549	0.5789	1.0000
Igf1	0.5989	0.5682	1.0000	1.8385	0.1028	0.9824
Inhba	0.1165	0.8919	1.0000	0.4264	0.6764	1.0000
Jun	2.9252	0.0163	0.5094	2.3881	0.0488	0.8416
Junb	3.4492	0.0112	0.2550	1.7831	0.1124	0.9844
Kif17	0.7673	0.4705	1.0000	1.7375	0.0732	0.9892
Klf10	0.4054	0.9439	1.0000	1.6209	0.1401	0.9979
Mapk1	1.7265	0.1161	0.9951	2.3917	0.0476	0.8416
Mmp9	0.1795	0.8204	1.0000	1.5424	0.1590	1.0000
Ncam1	0.5563	0.5870	1.0000	0.4349	0.6654	1.0000
Nfkb1	0.0849	0.9308	1.0000	2.4694	0.0394	0.7961
Nfkbib	0.4718	0.8003	1.0000	1.4319	0.1677	1.0000
Ngf	0.4270	0.6864	1.0000	0.9696	0.3674	1.0000
Ngfr	1.6174	0.1324	0.9988	0.1958	0.8670	1.0000
Nos1	1.1009	0.2948	1.0000	3.6820	**0.0020**	0.2644
Nptx2	0.0855	0.9418	1.0000	0.4755	0.6859	1.0000
Nr4a1	2.0433	0.0602	0.9512	0.8997	0.3835	1.0000

Gene	Adolescents			Adults		
	t-stat	p0Stat	pStat	t-stat	p0Stat	pStat
Ntf3	1.7555	0.1150	0.9939	1.7083	0.1262	0.9914
Ntf5	3.4003	**0.0084**	0.2729	1.0765	0.3277	1.0000
Ntrk2	0.0842	0.9387	1.0000	2.3997	0.0382	0.8304
Pcdh8	0.4915	0.6184	1.0000	1.3499	0.2075	1.0000
Pick1	0.5030	0.6207	1.0000	1.0454	0.3043	1.0000
Pim1	2.6424	0.0330	0.6717	4.2979	**0.0020**	0.1335
Plat	0.4053	0.7040	1.0000	2.8331	0.0174	0.6306
Plcg1	1.9893	0.0970	0.9605	2.5342	0.0452	0.7568
Ppp1ca	1.9932	0.0240	0.9592	2.4985	0.0392	0.7832
Ppp1cc	0.9188	0.3749	1.0000	1.2980	0.2305	1.0000
Ppp1r14a	2.6975	0.0198	0.6351	1.2661	0.2330	1.0000
Ppp2ca	1.3606	0.2038	1.0000	1.9850	0.0908	0.9652
Ppp3ca	0.8023	0.4532	1.0000	0.1437	0.9151	1.0000
Prkca	0.1026	0.9079	1.0000	0.6793	0.5004	1.0000
Prkcc	1.1744	0.2562	1.0000	1.0875	0.3078	1.0000
Prkg1	1.5265	0.1572	1.0000	2.4857	0.0252	0.7896
Rab3a	0.5568	0.5737	1.0000	0.0467	0.9651	1.0000
Rela	1.0270	0.3329	1.0000	2.0485	0.0707	0.9438
Reln	0.3049	0.7784	1.0000	2.1285	0.0558	0.9262
Rgs2	2.0380	0.0737	0.9525	1.7142	0.1238	0.9914
Rheb	0.2457	0.8134	1.0000	0.2203	0.8263	1.0000
Sirt1	0.3793	0.7021	1.0000	3.5587	0.0124	0.2997
Srf	1.4641	0.1667	1.0000	2.3053	0.0524	0.8783
Synpo	2.0075	0.0709	0.9562	0.5716	0.5791	1.0000
Timp1	1.7484	0.1157	0.9939	2.5558	0.0343	0.7485
Tnf	1.0193	0.3082	1.0000	3.6007	**0.0020**	0.2864
Ywhaq	1.6374	0.1302	0.9976	0.7420	0.4711	1.0000

Bold value indicates experiment-wise significance in permutation analysis.

Table 6

Symbol and description of the 84 synaptic plasticity genes in the array used in the current study

Position	Symbol	Description
A01	Adam10	A disintegrin and metallopeptidase domain 10
A02	Adcy1	Adenylate cyclase 1
A03	Adcy8	Adenylate cyclase 8
A04	Akt1	Thymoma viral proto-oncogene 1
A05	Arc	Activity regulated cytoskeletal-associated protein
A06	Bdnf	Brain derived neurotrophic factor
A07	Camk2a	Calcium/calmodulin-dependent protein kinase II alpha
A08	Camk2g	Calcium/calmodulin-dependent protein kinase II gamma
A09	Cdh2	Cadherin 2
A10	Cebpb	CCAAT/enhancer binding protein (C/EBP), beta
A11	Cebpd	CCAAT/enhancer binding protein (C/EBP), delta
A12	Cnr1	Cannabinoid receptor 1 (brain)
B01	Creb1	CAMP responsive element binding protein 1
B02	Crem	CAMP responsive element modulator
B03	Dlg4	Disks, large homolog 4 (Drosophila)
B04	Egr1	Early growth response 1
B05	Egr2	Early growth response 2
B06	Egr3	Early growth response 3
B07	Egr4	Early growth response 4
B08	Ephb2	Eph receptor B2
B09	Fos	FBJ osteosarcoma oncogene
B10	Gabra5	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 5
B11	Gnai1	Guanine nucleotide binding protein (G protein), alpha inhibiting 1
B12	Gria1	Glutamate receptor, ionotropic, AMPA1 (alpha 1)
C01	Gria2	Glutamate receptor, ionotropic, AMPA2 (alpha 2)
C02	Gria3	Glutamate receptor, ionotropic, AMPA3 (alpha 3)
C03	Gria4	Glutamate receptor, ionotropic, AMPA4 (alpha 4)
C04	Grin1	Glutamate receptor, ionotropic, NMDA1 (zeta 1)
C05	Grin2a	Glutamate receptor, ionotropic, NMDA2A (epsilon 1)
C06	Grin2b	Glutamate receptor, ionotropic, NMDA2B (epsilon 2)
C07	Grin2c	Glutamate receptor, ionotropic, NMDA2C (epsilon 3)
C08	Grin2d	Glutamate receptor, ionotropic, NMDA2D (epsilon 4)
C09	Grip1	Glutamate receptor interacting protein 1
C10	Grm1	Glutamate receptor, metabotropic 1
C11	Grm2	Glutamate receptor, metabotropic 2
C12	Grm3	Glutamate receptor, metabotropic 3
D01	Grm4	Glutamate receptor, metabotropic 4
D02	Grm5	Glutamate receptor, metabotropic 5
D03	Grm7	Glutamate receptor, metabotropic 7

Position	Symbol	Description
D04	Grm8	Glutamate receptor, metabotropic 8
D05	Homer1	Homer homolog 1 (Drosophila)
D06	Igf1	Insulin-like growth factor 1
D07	Inhba	Inhibin beta-A
D08	Jun	Jun oncogene
D09	Junb	Jun-B oncogene
D10	Kif17	Kinesin family member 17
D11	Klf10	Kruppel-like factor 10
D12	Mapk1	Mitogen-activated protein kinase 1
E01	Mmp9	Matrix metallopeptidase 9
E02	Ncam1	Neural cell adhesion molecule 1
E03	Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105
E04	Nfkbib	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta
E05	Ngf	Nerve growth factor
E06	Ngfr	Nerve growth factor receptor (TNFR superfamily, member 16)
E07	Nos1	Nitric oxide synthase 1, neuronal
E08	Nptx2	Neuronal pentraxin 2
E09	Nr4a1	Nuclear receptor subfamily 4, group A, member 1
E10	Ntf3	Neurotrophin 3
E11	Ntf5	Neurotrophin 5
E12	Ntrk2	Neurotrophic tyrosine kinase, receptor, type 2
F01	Pcdh8	Protocadherin 8
F02	Pick1	Protein interacting with C kinase 1
F03	Pim1	Proviral integration site 1
F04	Plat	Plasminogen activator, tissue
F05	Plcg1	Phospholipase C, gamma 1
F06	Ppp1ca	Protein phosphatase 1, catalytic subunit, alpha isoform
F07	Ppp1cc	Protein phosphatase 1, catalytic subunit, gamma isoform
F08	Ppp1r14a	Protein phosphatase 1, regulatory (inhibitor) subunit 14A
F09	Ppp2ca	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform
F10	Ppp3ca	Protein phosphatase 3, catalytic subunit, alpha isoform
F11	Prkca	Protein kinase C, alpha
F12	Prkcc	Protein kinase C, gamma
G01	Prkg1	Protein kinase, cGMP-dependent, type I
G02	Rab3a	RAB3A, member RAS oncogene family
G03	Rela	V-rel reticuloendotheliosis viral oncogene homolog A (avian)
G04	Reln	Reelin
G05	Rgs2	Regulator of G-protein signaling 2
G06	Rheb	Ras homolog enriched in brain
G07	Sirt1	Sirtuin 1 (silent mating type information regulation 2, homolog) 1 (S. cerevisiae)
G08	Srf	Serum response factor
G09	Synpo	Synaptopodin

Position	Symbol	Description
G10	Timp1	Tissue inhibitor of metalloproteinase 1
G11	Tnf	Tumor necrosis factor
G12	Ywhaq	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide