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Csnk1e Is a Genetic Regulator of Sensitivity to Psychostimulants and Opioids

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Csnk1e, the gene encoding casein kinase 1-epsilon, has been implicated in sensitivity to amphetamines. Additionally, a polymorphism in *CSNK1E* was associated with heroin addiction, suggesting that this gene may also affect opioid sensitivity. In this study, we first conducted genome-wide quantitative trait locus (QTL) mapping of methamphetamine (MA)-induced locomotor activity in C57BL/6J (B6) × DBA/2J (D2)-F_2 mice and a more highly recombinant F_8 advanced intercross line. We identified a QTL on chromosome 15 that contained *Csnk1e* (63–86 Mb; *Csnk1e* = 79.25 Mb). We replicated this result and further narrowed the locus using B6.D2^{Csnk1e} and D2.B6^{Csnk1e} reciprocal congenic lines (78–86.8 and 78.7–81.6 Mb, respectively). This locus also affected sensitivity to the μ-opioid receptor agonist fentanyl. Next, we directly tested the hypothesis that *Csnk1e* is a genetic regulator of sensitivity to psychostimulants and opioids. Mice harboring a null allele of *Csnk1e* showed an increase in locomotor activity following MA administration. Consistent with this result, coadministration of a selective pharmacological inhibitor of Csnk1e (PF-4800567) increased the locomotor stimulant response to both MA and fentanyl. These results show that a narrow genetic locus that contains *Csnk1e* is associated with differences in sensitivity to MA and fentanyl. Furthermore, gene knockout and selective pharmacological inhibition of Csnk1e define its role as a negative regulator of sensitivity to psychostimulants and opioids.
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INTRODUCTION

Psychostimulants and opioids increase locomotor activity in rodents in part via dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988; Wise and Bozarth, 1987), an important brain area for reward learning (Flagel *et al*, 2011). Variation in sensitivity to the locomotor stimulant effect of amphetamines and opioids is heritable (Belknap *et al*, 1998; Phillips *et al*, 2008; Gill and Boyle, 2008; Oliverio *et al*, 1975; Philip *et al*, 2010). Because epidemiological studies indicate that sensitivity to drug liking in humans can predict an individual's susceptibility to drug abuse (Haertzen *et al*, 1983; Schuckit, 2009), drug sensitivity and abuse are hypothesized to share a genetic basis. This hypothesis is supported by genetic association studies in humans that have identified genes that influence both acute sensitivity (Hamidovic *et al*, 2009, 2010; Lott *et al*, 2005, 2006; Dlugos *et al*, 2011) and dependence (Ho *et al*, 2010).

We previously identified a quantitative trait locus (QTL) for methamphetamine (MA) sensitivity on chromosome 15 near *Csnk1e* (Palmer *et al*, 2005), the gene encoding casein kinase 1-epsilon. *Csnk1e* is a member of the casein kinase-1 (CK-1) family of serine/threonine kinases (Cheong and Virshup, 2010). CK-1 phosphorylates dopamine- and cyclic adenosine monophosphate-regulated neuronal phosphoprotein-32 (DARPP-32) (Greengard, 2001), which regulates psychostimulant- and opioid-induced locomotor activity (Borgkvist and Fisone, 2007; Borgkvist *et al*, 2007). CK-1 also phosphorylates *per* proteins (Meng *et al*, 2008) that regulate gene transcription in circadian rhythms and the molecular and behavioral adaptations to psychostimulants (Falcon and McClung, 2008). Last, CK-1 phosphorylates

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various proteins of the canonical Wnt signaling pathway and may contribute to neuropsychiatric conditions such as mood disorders (Li and Jope, 2010).

We previously showed that the *CSNK1E* SNP rs135745 was associated with amphetamine-induced subjective euphoria in healthy human volunteers (Veenstra-VanderWeele *et al*, 2006) and have since replicated this finding in a new cohort of 282 subjects (Amy *et al*, unpublished data). Additionally, the *CSNK1E* SNP rs1534891 was associated with heroin addiction (Levran *et al*, 2008). Furthermore, coadministration of the casein kinase 1-δ (Csnk1d)-preferring inhibitor PF-670462 with MA decreased locomotor activity and DARPP-32 phosphorylation in mice (Bryant *et al*, 2009b), and a similar result was observed following coadministration of PF-670462 with amphetamine in rats (Li *et al*, 2011). Conversely, Csnk1d overexpression in the forebrain resulted in baseline hyperactivity and an increased locomotor response to amphetamine (Zhou *et al*, 2010). Together, these results suggest that both Csnk1e and Csnk1d are important for sensitivity to drugs of abuse. However, because PF-670462 only exhibits six-fold selectivity for Csnk1d over Csnk1e (Walton *et al*, 2009; Meng *et al*, 2010), it is unclear if our previous results were mediated by Csnk1d or Csnk1e (Bryant *et al*, 2009b; Li *et al*, 2011).

In this study, we employed a forward genetic approach using F_2 and F_8 advanced intercross mice as well as reciprocal congenic lines derived from the C57BL/6J (B6) and DBA/2 J (D2) strains to fine map a QTL that influences MA sensitivity (Palmer *et al*, 2005). Because we previously observed a higher D2 allelic frequency at the *Csnk1e* locus in a mouse line selected for high MA sensitivity (Palmer *et al*, 2005), we hypothesized that the D2 allele would increase drug sensitivity. Based on the location of our QTL and prior studies implicating *Csnk1e* in the response to drugs of abuse, we further hypothesized that *Csnk1e* regulates sensitivity to psychostimulants and opioids. Thus, we examined the effect of a *Csnk1e*-null mutation or pharmacological inhibition with the new selective Csnk1e inhibitor PF-4800567 (Walton *et al*, 2009; Meng *et al*, 2010) on the locomotor response to MA and the μ-opioid receptor agonist fentanyl.

MATERIALS AND METHODS

Environment and Housing

All experiments were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by IACUC at the University of Chicago and Northwestern University. Mouse colony rooms were maintained on a 12/12 light/dark cycle with lights on at 0600 h. Two to five same-sex littermates were housed in clear plastic shoe box–sized cages. Mice were allowed *ad libitum* access to food and water, except during testing. An approximately equal number of female and male mice were employed for each genotype/treatment group for each study.

B6 $\times$ D2-F_2 and -F_8 Advanced Intercross Mice

Inbred female B6 and male D2 mice were obtained from The Jackson Laboratory (JAX; Bar Harbor, ME). These mice were used to produce B6 $\times$ D2 F_1 mice, which were then intercrossed to create the F_2 ($N = 676$) and F_8 ($N = 552$) generations. F_8 breeding was pseudorandom to ensure that siblings and close relatives were not mated with one another. Additionally, breeding pairs were rotated after each litter to avoid producing large numbers of full sibs. However, the F_8 generation inevitably contained many sibs, half-sibs, and cousins as well as more distant and complex relationships that were accounted for in the analysis. F_2 and F_8 mice were 7–13 weeks old at the time of testing.

For genotyping, DNA from the F_2 generation was extracted and genotyped by KBiosciences (Hoddesdon, Hertfordshire, UK) using KASPar, a fluorescence-based PCR assay. Markers consisted of 164 evenly spaced, informative SNPs that were selected from a previous study (Petkov *et al*, 2004). In the F_8 generation, DNA was extracted and then genotyped using the Illumina Mouse Medium Density Linkage Panel (Illumina, San Diego, CA) at the Genomics Core Facility at Northwestern University (http://web.cgm.northwestern.edu/cgm/Core-Facilities/Genomics-Core). The SNP array consisted of 1449 loci. Of these, 909 were polymorphic between B6 and D2 mice and 882 of these markers were used in this study.

B6.D2^{Csnk1e} and D2.B6^{Csnk1e} Reciprocal Congenic Mice

B6.D2^{Csnk1e} mice were derived from a larger congenic line that was previously backcrossed to B6 (JAX) for more than 10 generations (Iakoubova *et al*, 2001). D2.B6^{Csnk1e} mice were derived from a larger congenic line at the Wadsworth Center (Albany, NY) that was previously backcrossed to D2 (JAX) for more than 10 generations. We further backcrossed both congenic lines to their respective background strains until we obtained smaller congenic regions capturing the *Csnk1e* locus and then intercrossed (heterozygous–heterozygous breeding) to yield congenics and wild-type littermates for simultaneous phenotyping. Because the inhibitor study required a large number of mice, we used homozygous–homozygous breeders chosen from the offspring of heterozygous–heterozygous breeders and from a single generation of inbreeding. Congenic mice were 7–12 weeks old at the time of testing.

For genotyping, we first used a series of TaqMan SNP markers on chromosome 15 from Applied Biosystems (Foster City, CA) to monitor recombination events. In order to precisely determine the congenic boundaries, we then genotyped SNPs chosen from the Mouse Phenome Database (http://phenome.jax.org/) using PCR amplification, gel extraction, and DNA Sanger sequencing at the University of Chicago core facility (http://cancer-seqbase.uchicago.edu/). We then re-genotyped two mice from each line on the same SNP array as described above to reveal any regions of residual heterozygosity (RH). RH SNPs were confirmed via DNA sequencing from the same individuals.

Csnk1e Knockout Mice

Csnk1e knockout mice were generated as previously described (Meng *et al*, 2008). *Csnk1e* knockout mice were backcrossed for six generations to C57BL/6N mice. Heterozygous–heterozygous breeding was used to generate the first cohort of mice for testing (27 offspring) followed by

homozygous–homozygous breeding of a single generation of breeders for the second cohort of mice (27 offspring), equaling a total of 54 mice (26 *Csnk1e* wild types and 28 *Csnk1e* knockouts). Mice were 8–21 weeks old at the time of testing. Approximately equal numbers of age-matched wild types and knockouts were always tested within the same experimental session. Genotyping was conducted via PCR and gel electrophoresis and SYBR green staining using primers (5′-CGGGAAAACAAGAACCTGAC-3′) and (5′-TAGGTCATCTCGACGGCTTT-3′) to generate a 750 bp band for the wild-type allele and using primers (5′-CGGCTC AGTGATGGGTACT-3′) and (5′-TAGGTCATCTCGACGGCT TT-3′) to generate a 730 bp band for the null allele.

Drugs

Methamphetamine HCl (MA; Sigma-Aldrich, St Louis, MO; 2 mg/kg, i.p.) and fentanyl citrate (Sigma-Aldrich; 0.2 mg/kg, i.p.) were dissolved in 0.9% NaCl. The dose of MA was chosen based on our prior QTL mapping and pharmacological studies (Palmer *et al*, 2005; Bryant *et al*, 2009b). The dose of fentanyl was chosen based on our previous studies employing this dose in B6 mice in the open field (Bryant *et al*, 2009a, 2009c). PF-4800567 (PF; 40 mg/kg, i.p.) was dissolved in a 10% v-v solution of 1,2-propanediol (polypropylene glycol)/double deionized water containing 30% (w-v) sulfobutylether-β-cyclodextrin (Captisol (CyDex Pharmaceuticals, Lenexa, KS)). The polypropylene glycol/PF solution was briefly sonicated using a Qsonica Misonix S-4000 (Farmingdale, NY) before adding 30% Captisol solution. The dose of PF was chosen based on previously published studies in mice (Walton *et al*, 2009; Meng *et al*, 2010), a limit in solubility at higher concentrations, and pilot studies indicating its effectiveness at modulating drug-induced locomotor activity. PF has been shown to exhibit 20-fold greater selectivity for Csnk1e *vs* Csnk1d and has been useful in clarifying the importance of the two isoforms in regulating circadian rhythms (Walton *et al*, 2009; Meng *et al*, 2010).

Locomotor Testing Procedure

Testing was conducted between 0800 h and 1600 h. Mice were transported from the adjacent vivarium into the test room and were allowed to habituate for at least 30 min before testing. Just before testing, mice were removed from their home cages and placed in clean holding cages for $\sim$5 min and then received an intraperitoneal (i.p.) injection of saline (10 ml/kg) on days 1 and 2 and either MA (2 mg/kg, i.p) or saline (i.p.) or fentanyl (0.2 mg/kg, i.p.) on day 3. Mice were then immediately placed in the center of the open field and that total distance traveled was recorded in 5-min bins over 30 min. Locomotor activity was measured using the automated Versamax activity chambers (AccuScan, Columbus, OH; F_2, F_8, and congenic mice at University of Chicago) or Big Brother software (Coulbourn Instruments, Whitehall, PA; *Csnk1e* knockout and wild-type mice at Northwestern University). The open field was 37.5 $\times$ 37.5 $\times$ 35.7 cm in size. For Versamax, beam breaks were recorded on a computer and converted into total distance traveled (cm). For Big Brother, the dimensions of the image width of the open field were used to convert locomotor activity into total distance traveled (cm) that was recorded on a computer.

Inhibitor Study

When comparing the vehicle solution *vs* saline in pilot studies, there was a progressive decrease in locomotor activity following two vehicle exposures on days 1 and 2. This could be caused both by a larger injection volume and by the fact that one of the components of the vehicle solution, polypropylene glycol, has been shown to decrease locomotor activity at high concentrations (Singh *et al*, 1982). We controlled for potential effects of vehicle across treatment groups by including both components of the vehicle (polypropylene glycol plus Captisol) in the control solution that did not contain PF. Additionally, to limit the amount of vehicle exposure, we chose to administer saline during habituation on days 1 and 2 and to only administer the vehicle solution on day 3 (containing PF or vehicle alone). On day 3, mice were injected with either vehicle (20 ml/kg, i.p., left cavity) or PF (40 mg/kg, i.p., 20 ml/kg, left cavity) followed immediately thereafter with saline (10 ml/kg, i.p.; right cavity), MA (2 mg/kg, i.p., 10 ml/kg, right cavity), or fentanyl (0.2 mg/kg, i.p., 10 ml/kg, right cavity). Mice were then placed into the open field and the total distance traveled was recorded over 30 min.

QTL Analysis

For QTL analysis, kinship coefficients and identity coefficients were calculated using the pedigree for the AIL, which began in the F_1 and continued to the F_8 generation as previously described (Cheng *et al*, 2010; Parker *et al*, 2011). Genome-wide association analysis was performed in the combined population of the F_2 and F_8 intercrosses using the R package QTLRel (Cheng *et al*, 2011). This software accounts for the complex relationships (eg, sibling, half-sibling, cousins) among F_8 mice by using a mixed model. In addition, genetic models where sex was included as an additive or interactive covariate were explored; however, these models did not affect the results and thus the sexes were combined for the final analysis. For each analysis, $P<0.05$ significance thresholds were determined via permutation analysis (1000 permutations).

Behavioral Analysis

We used the difference in locomotor activity between days 2 and 3 (day 3–day 2) as the primary measure of drug sensitivity for the QTL and congenic analysis (Palmer *et al*, 2005). This measure takes into account differences in locomotor activity that are not specific to drug treatment. For the inhibitor study, we used day 3 activity as the measure of drug sensitivity because the vehicle for PF was only administered on day 3 and not day 2. For all behavioral studies, we first ran a repeated measures ANOVA (genotype and sex as factors; time as repeated measure) and determined that sex did not interact with genotype. Thus, the sexes were combined and repeated measures ANOVA followed by the indicated *post-hoc* test were employed to determine the main effects, interactions, and their source.

RESULTS

QTL

We identified a QTL on chromosome 15 for day 3–day 2 activity following MA administration that peaked at 5 min and reached genome-wide significance (LOD = 5.18; $P<0.05$ significance threshold = 4.01). This QTL was not significant at 10 or 15 min (Figure 1a). The 1.5-LOD support interval ranged from 63 to 86 Mb, thus capturing *Csnk1e* (79.25–79.27 Mb). The D2 allele of the marker nearest the peak LOD score was associated with higher MA sensitivity in both the F_2 ($t_{274} = 3.78$; $P<0.05$) and F_8 cross ($t_{282} = 2.36$; $P<0.05$; Figure 1b and c). We identified other genome-wide significant QTLs that are the subject of a separate investigation (Parker *et al*, 2011a, under review).

Reciprocal Congenic Regions

The congenic boundaries are illustrated in Figure 2a and b and documented in detail in Supplementary Table S1. The introgressed region of the B6.D2^{Csnk1e} line spanned 78–86.8 Mb whereas for the D2.B6^{Csnk1e} line spanned 78.7–81.6 Mb. The latter region was estimated to be ~0.56 cM (Cox *et al*, 2009). As expected, the results of the SNP array confirmed that each congenic line contained <1% RH. Specifically, for B6.D2^{Csnk1e}, one individual did not harbor any RH whereas the second individual harbored RH at only 2 markers out of 882. These SNPs were located on chromosome 11 (rs13480863; 9.19 Mb) and chromosome 13 (rs3663223; 23.18 Mb) and were confirmed via PCR and DNA sequencing of the region harboring the SNP. Neither of these RH loci resided in the two individuals tested from the D2.B6^{Csnk1e} line.

With respect to D2.B6^{Csnk1e} mice, one individual did not harbor any RH, whereas the second individual harbored RH at 6 out of 882 markers (confirmed via PCR and DNA sequencing). In sum, the SNP array results indicate that RH in the congenic lines was very limited and was <1%. There was no pattern of RH within a line and RH resided in different loci between lines. As such, it is highly unlikely that RH was responsible for the predicted reciprocal effect on MA-induced locomotor activity. The most parsimonious explanation is that the region on chromosome 15 that mediates the QTL is the common denominator responsible for the expected change in phenotype across the congenic lines.

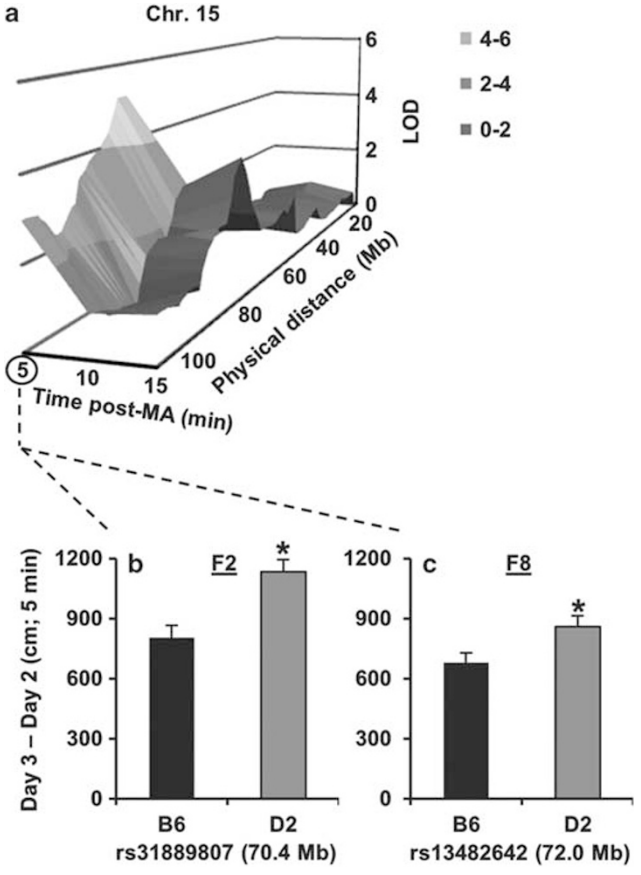

Figure 1 Chromosome 15 QTL for MA sensitivity in B6 × D2 -F_2 and -F_8 mice. (a) Three-dimensional plot of LOD score *vs* time after MA (2 mg/kg, i.p.) *vs* physical position (Mb) of the SNP markers. The 1.5-LOD support interval was 63–86 Mb. (b, c) The D2 allele at the marker nearest the peak LOD was associated with an increase in MA sensitivity relative to the B6 allele in F_2 and F_8 mice. Data for each genotype are presented as the average ± SEM. *$P<0.05$.

MA sensitivity in B6.D2^{Csnk1e} and D2.B6^{Csnk1e} Reciprocal Congenic Mice

In examining the response to MA in B6.D2^{Csnk1e} mice, there was a main effect of genotype ($F_{1,64} = 5.59$; $P<0.05$) and time ($F_{2,128} = 75.15$; $P<0.05$). B6.D2^{Csnk1e} mice showed significantly greater MA-induced locomotor activity than B6.B6^{Csnk1e} mice at 5 min ($t_{64} = 3.94$; $P<0.05$; Figure 2c) but not at 10 or 15 min ($P>0.05$). D2.B6^{Csnk1e} mice demonstrated significantly less MA-induced locomotor activity than D2.D2^{Csnk1e} mice at 5 min ($t_{88} = 2.32$; $P<0.05$; Figure 2d) but not at 10 or 15 min ($P>0.05$). In sum, B6.D2^{Csnk1e} and D2.B6^{Csnk1e} mice exhibit the hypothesized reciprocal, time-dependent changes in MA sensitivity based on our previous observations (Palmer *et al*, 2005) and the QTL mapping results shown in Figure 1.

Saline Control Experiment in B6.D2^{Csnk1e} and D2.B6 ^{Csnk1e} Reciprocal Congenic Mice

We performed a control experiment to ensure that the effect of the congenic region on MA-induced locomotor activity was specific to drug treatment and not explained by differences in drug-free activity between days 2 and 3. Because we wanted to know how confidently we could interpret any negative results, we first conducted a power analysis to determine the required sample size for detecting an effect of genotype on locomotor activity. Based on the means and standard deviations of the two genotypes, we calculated the effect size *r*. We then converted these values to Cohen's *d* and determined the sample size required to detect a significant difference of the two groups using G-Power 3.1 (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/). The estimated effect size for the difference in MA-induced locomotor activity in B6.D2^{Csnk1e} mice is $r = 0.44$. Thus, $N = 14$ per genotype is required to detect this difference with $\alpha = 0.05$ and 80% power. The estimated effect size for the difference in MA-induced locomotor

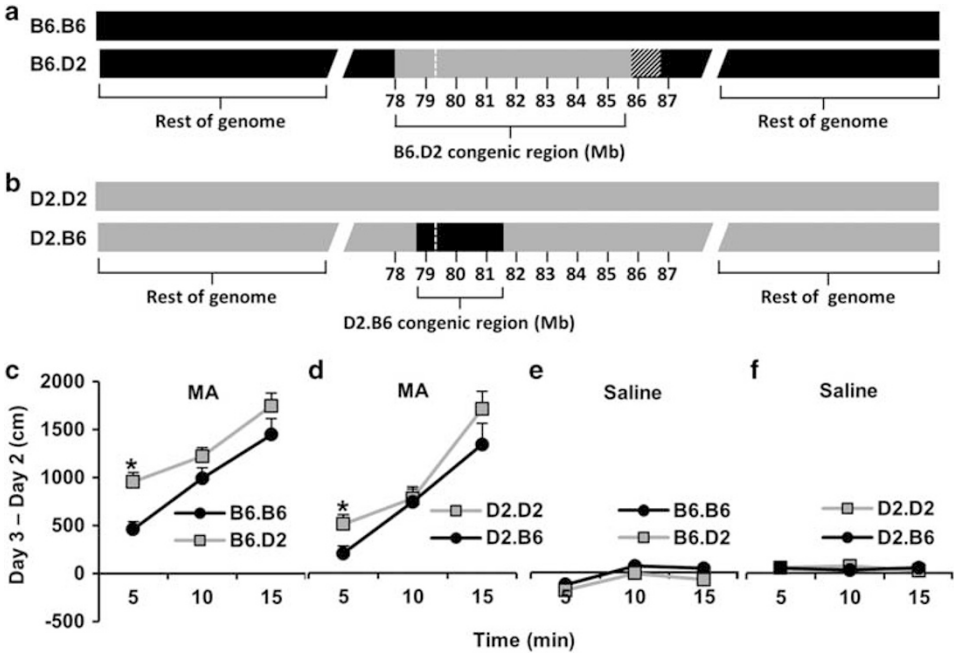

Figure 2 B6.D2^{Csnk1e} and D2.B6 ^{Csnk1e} reciprocal congenic regions and MA sensitivity. (a) The B6.D2^{Csnk1e} congenic line contained an introgressed region on chromosome 15 (gray) spanning 78–86.8 Mb. (b) The D2.B6^{Csnk1e} congenic line contained a smaller introgressed region (black) spanning 78.7–81.6 Mb (see Supplementary Table S1 for additional details). 'Rest of genome' is >99% homozygous for the respective background strain (black for B6, gray for D2) at genomic loci outside of the congenic regions. The diagonally striped region was not genotyped. The physical position of *Csnk1e* is 79.25 Mb (Dotted, white vertical line). (c, d) Day 3–day 2 activity is shown for B6.D2^{Csnk1e} mice ($N=32$) *vs* their B6.B6 wild-type littermates ($N=34$) and for D2.B6^{Csnk1e} mice ($N=39$) *vs* their D2.D2^{Csnk1e} wild-type littermates ($N=51$) in response to MA (2 mg/kg, i.p.) on day 3. (e, f) Day 3–day 2 activity is shown for B6.D2^{Csnk1e} ($N=23$) *vs* their B6.B6^{Csnk1e} wild-type littermates ($N=15$) and for D2.B6^{Csnk1e} ($N=40$) *vs* their D2.D2^{Csnk1e} wild-type littermates ($N=40$) in response to saline (i.p.) on day 3. Data for each genotype are presented as the average ± SEM for each 5 min bin. *$P<0.05$.

activity in D2.B6^{Csnk1e} mice is $r=0.25$. Thus, $N=49$ per genotype is required to detect this difference.

Based on the power analysis, a separate cohort of congenic mice and their wild-type littermates received saline on day 3 instead of MA. In B6.D2^{Csnk1e} mice ($N=23$) *vs* B6.B6^{Csnk1e} mice ($N=15$), there was no effect of genotype ($P>0.05$). We confirmed this negative result by examining the first 5 min ($P>0.05$; Figure 2e). With regard to D2.B6^{Csnk1e} mice ($N=40$) *vs* D2.D2^{Csnk1e} mice ($N=40$), there was no effect of genotype or an interaction with time; this negative result was also confirmed by examining the first 5 min ($P>0.05$; Figure 2f). Thus, the congenic phenotype is specific to MA treatment and cannot be explained by drug-free differences in locomotor activity between days 2 and 3.

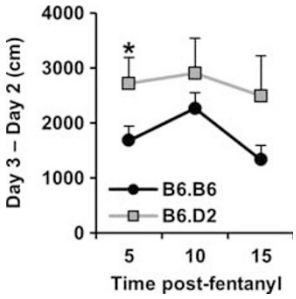

Figure 3 Fentanyl sensitivity in B6.D2^{Csnk1e} mice. Day 3–day 2 activity is shown for B6.D2^{Csnk1e} mice ($N=11$) *vs* their B6.B6^{Csnk1e} wild-type littermates ($N=16$) in response to fentanyl (0.2 mg/kg, i.p.) on day 3. Data for each genotype are presented as the average ± SEM for each 5 min bin. *$P<0.05$.

Fentanyl Sensitivity in B6.D2^{Csnk1e} Mice

It is well documented that the D2 inbred strain is resistant to the locomotor stimulant effects of opioid agonists, including morphine (Belknap *et al*, 1989, 1998; Orsini *et al*, 2005; Fadda *et al*, 2005; Cunningham *et al*, 1992; Gwynn and Domino, 1984; Hynes and Berkowitz, 1982; Collins and Whitney, 1978; Brase *et al*, 1977), heroin (Bailey *et al*, 2010; Castellano *et al*, 1976), and methadone (Middaugh and Zemp, 1976). We confirmed this lack of sensitivity to fentanyl-induced locomotor activity in D2 mice, and thus only examined fentanyl sensitivity in B6.D2^{Csnk1e} mice and their B6.B6^{Csnk1e} wild-type littermates. As predicted based on the MA results, B6.D2^{Csnk1e} mice showed significantly greater fentanyl-induced locomotor activity than B6.B6^{Csnk1e} mice at 5 min ($t_{25}=2.09$; $P<0.05$; Figure 3) but not at 10 or 15 min ($P>0.05$).

MA Sensitivity in *Csnk1e* Knockout Mice

For day 1, there was no effect of genotype and no genotype × time interaction ($P>0.05$; data not shown). For day 2, there was an effect of genotype ($F_{1,52}=6.02$; $P<0.05$). *Csnk1e* knockout mice showed greater activity in response to a saline injection than *Csnk1e* wild-type mice at 10, 15, 20, and 25 min ($t_{52}=3.12$, 2.34, 2.67, and 2.02; $P<0.05$; Figure 4a). On day 3, in response to MA, there was

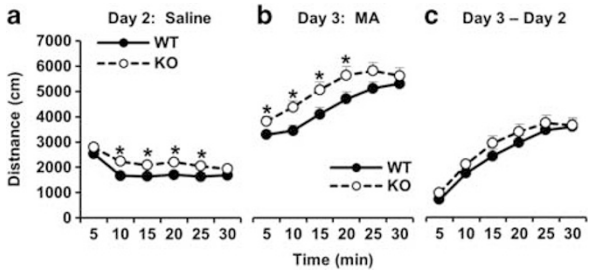

Figure 4 MA sensitivity in *Csnk1e* knockout mice. (a) Locomotor activity on day 2 in response to saline (i.p.) in *Csnk1e* knockout (KO) mice ($N=28$) *vs Csnk1e* wild-type (WT) mice ($N=26$). (b) Locomotor activity on day 3 in response to MA (2 mg/kg, i.p.). (c) Day 3–day 2 activity. Data for each genotype are presented as the average ± SEM for each 5 min bin. $^*P<0.05$.

an effect of genotype ($F_{1,52}=4.13$; $P<0.05$) and an interaction with time ($F_{5,260}=2.69$; $P<0.05$). *Csnk1e* knockout mice showed greater MA-induced locomotor activity at 5, 10, 15, and 20 min ($t_{52}=2.19$, 2.70, 2.29, and 2.07; $P<0.05$; Figure 4b). For day 3–day 2, there was no effect of genotype or interaction with time ($P>0.05$; Figure 4c). Because the age range of mice used in this experiment was wide (8–21 weeks), we used a median split and divided the mice into a younger group and an older group and added 'age group' as an additional factor to the repeated measures ANOVA. The effect of genotype remained significant ($P<0.05$), there was no effect of age group ($P>0.05$), and there was no genotype × age group interaction ($P>0.05$) for both days 2 and 3 (data not shown).

Effect of PF-4800567 on MA and Fentanyl Sensitivity in B6.B6^{Csnk1e} and B6.D2^{Csnk1e} Mice

In examining the effect of PF on MA sensitivity in B6.B6^{Csnk1e} mice, there was no effect of treatment ($P>0.05$; Figure 5a); however, in B6.D2^{Csnk1e} mice, there was an effect of treatment ($F_{1,35}=4.34$; $P<0.05$) whereby mice receiving PF showed significantly greater MA-induced locomotor activity than mice receiving vehicle over 30 min (Figure 5b). The effect of PF on fentanyl-induced locomotor stimulation was more robust and was observed in both genotypes. In B6.B6^{Csnk1e} mice, there was an effect of treatment ($F_{1,33}=9.07$; $P<0.05$) and an interaction with time ($F_{5,165}=2.63$; $P<0.05$) that was explained by PF-treated mice exhibiting greater fentanyl-induced locomotor activity than vehicle-treated mice at 5, 15, 20, 25, and 30 min ($t_{33}=2.23$, 3.07, 3.17, 2.83, and 2.59; $P<0.05$; Figure 5c). In B6.D2^{Csnk1e} mice, there was a main effect of treatment ($F_{1,28}=6.16$; $P<0.05$) and an interaction with time ($F_{5,140}=3.45$; $P<0.05$) that was explained by PF-treated mice showing significantly greater fentanyl-induced locomotor activity than vehicle-treated mice at 15, 20, 25, and 30 min ($t_{28}=2.44$, 3.05, 2.46, and 2.83; $P<0.05$; Figure 5d). In this study, we did not observe an effect of genotype during the first 5 min and B6.D2^{Csnk1e} mice tended to show less fentanyl activity. We attribute these observations to environmental differences (either in the home cage or during testing) because the offspring came from homozygote breeders and because the genotypes could not always be tested on the same day. Importantly, it does not affect the overall conclusion that pharmacological inhibition of Csnk1e increased sensitivity to both MA and fentanyl.

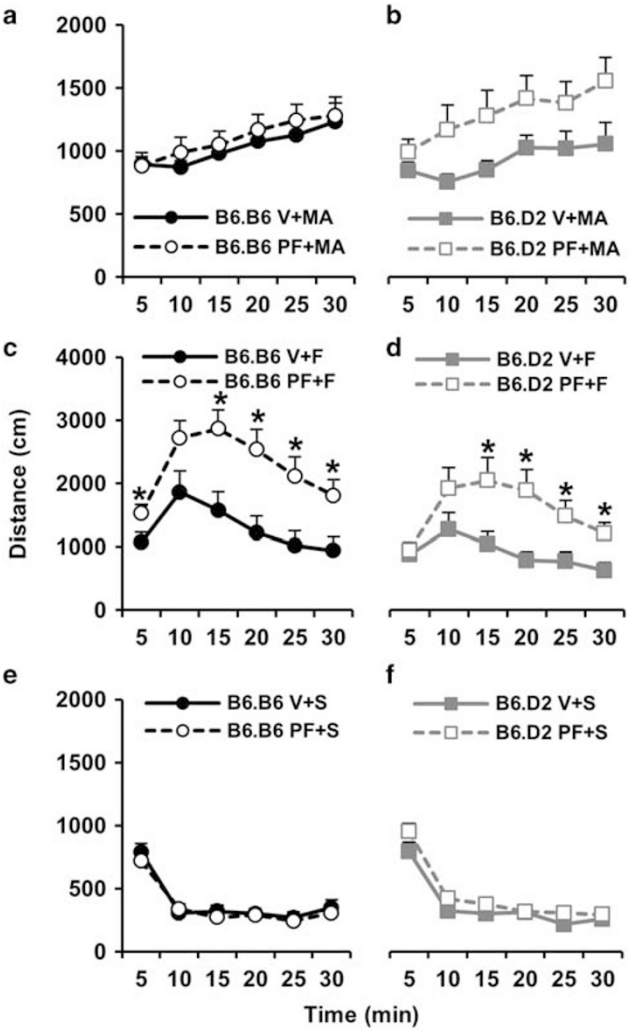

Figure 5 Effect of the Csnk1e inhibitor PF-4800567 on MA and fentanyl sensitivity in B6.B6^{Csnk1e} and B6.D2^{Csnk1e} mice. (a, b) Effect of PF-4800567 (PF; 40 mg/kg, i.p.) on MA-induced locomotor activity in B6.B6^{Csnk1e} mice ($N=18$ vehicle (V)-treated, $N=19$ PF-treated mice) and B6.D2^{Csnk1e} mice ($N=18$ V-treated, $N=20$ PF-treated mice). (c, d) Effect of PF on fentanyl (F)-induced locomotor activity in B6.B6^{Csnk1e} ($N=17$ V-treated, $N=18$ PF-treated mice) and B6.D2^{Csnk1e} mice ($N=15$ V-treated, $N=15$ PF-treated mice). (e, f) Effect of PF on saline (S)-induced locomotor activity in B6.B6^{Csnk1e} ($N=18$ V-treated, $N=17$ PF-treated mice) and B6.D2^{Csnk1e} mice ($N=17$ V-treated, $N=16$ PF-treated mice). Data for each genotype/treatment are presented as the average ± SEM for each 5 min bin. $^*P<0.05$.

Saline Control Experiment of PF-4800567 on Locomotor Activity in B6.B6^{Csnk1e} and B6.D2^{Csnk1e} Mice

In order to rule out the possibility that the enhancement of fentanyl-induced locomotor activity was mediated by a nonspecific increase in locomotor activity by PF, we ran a separate control experiment where PF was administered with saline (PF + S) and compared with a second group receiving vehicle plus saline (V + S). We first conducted a power analysis to determine the required sample size for

detecting an effect of the inhibitor on fentanyl-induced locomotor activity ($\alpha = 0.05$; 80% power). The effect size in B6.B6^{Csnk1e} mice is $r = 0.45$ and the required sample size is $N = 13$. The effect size in B6.D2^{Csnk1e} mice is $r = 0.41$ and the required sample size is $N = 16$. Following PF treatment alone, there was no effect of treatment or interaction with time in B6.B6 mice (Figure 5e; $N = 18$ vehicle-treated, $N = 17$ PF-treated mice; $P > 0.05$) or in B6.D2 mice (Figure 5f; $N = 17$ vehicle-treated, $N = 16$ PF-treated mice; $P > 0.05$), demonstrating that the enhancement of psychostimulant and opioid sensitivity by PF depends on their locomotor stimulant properties.

DISCUSSION

Using QTL and congenic analysis, we localized a small region on chromosome 15 flanking *Csnk1e* that modulates MA sensitivity (78.7–81.6 Mb; Figures 1 and 2). As predicted from our previous study using selected lines (Palmer *et al*, 2005), the D2 allele increased sensitivity to MA (Figures 1b and c and 2c and d). The *Csnk1e* locus regulated MA sensitivity in a time-dependent manner (Figure 1a) and, remarkably, the results of reciprocal congenic mice showed identical time dependence (Figure 2c and d). Thus, several independent lines of genetic evidence suggest that variation at the *Csnk1e* locus can regulate the locomotor response to MA, even when placed on different genetic backgrounds (F_2/F_8, B6, and D2). Interestingly, we recently replicated the association of the *CSNK1E* SNP rs135745 with amphetamine-induced subjective euphoria (Veenstra-VanderWeele *et al* 2006) in healthy human volunteers in a new cohort of 282 subjects (Amy *et al*, unpublished data).

The *Csnk1e* locus also affected opioid sensitivity (Figure 3) which, along with a previous association between a SNP in *CSNK1E* and heroin dependence (Levran *et al*, 2008), further implicates *Csnk1e* as a genetic regulator of sensitivity to drugs of abuse. Of particular note, a nearly identical genetic locus that we have identified (77–83 Mb *vs* 79–82 Mb in the present study) also regulates alcohol consumption in mice (Boyle and Gill, 2008). The convergence of a QTL on this region for the response to psychostimulants, opioids, and ethanol implicates gene(s) acting via dopaminergic mechanisms (Di Chiara and Imperato, 1988). Last, genetic (Figure 4b) and pharmacological (Figure 5b) inhibition of *Csnk1e* increased MA sensitivity and opioid sensitivity (Figure 5c and d). Together, these results suggest that genetic variation in *Csnk1e* modulates sensitivity to drugs of abuse and define a specific role for *Csnk1e* in negatively regulating sensitivity to both psychostimulants and opioids.

The effects of both the B6.D2^{Csnk1e} and D2.B6^{Csnk1e} intervals were specific to the locomotor stimulant properties of MA and fentanyl because there was no effect on locomotor activity following saline treatment (Figure 2e and f). Similarly, the selective Csnk1e inhibitor PF-4800567 increased both MA and fentanyl sensitivity (Figure 5b–d) while having no effect on locomotor activity following saline treatment (Figure 5e and f). PF-4800567 shows >20-fold selectivity for Csnk1e over Csnk1d and exhibits markedly reduced efficacy at inhibiting off-target proteins relative to PF-670462. Nevertheless, both PF-4800567 and PF-670462 can significantly inhibit epidermal growth factor receptor (EGFR) at micromolar concentrations (Walton *et al*, 2009). We are unaware of any study examining the acute effect of selective inhibition of EGFR on psychostimulant- or opioid-induced locomotor activity. Emodin, a nonselective compound that inhibits EGFR signaling (and ERK1/2 signaling among others) caused a significant reduction in the locomotor response to MA (Mizuno *et al*, 2008, 2010). However, these results are not consistent with the effect that PF-4800567 had on MA- and fentanyl-induced locomotor activity, which was an increase and not a decrease in locomotor activity.

Csnk1e knockout mice exhibited a significant increase in locomotor activity in both the absence and presence of MA (Figure 4a and b). Interestingly, this phenotype is very similar to what was observed following overexpression of *Csnk1d* in the forebrain: baseline hyperactivity that persisted following the administration of amphetamine (Zhou *et al*, 2010). Together, the results of these reverse genetic manipulations suggest that *Csnk1e* has an inhibitory role and *Csnk1d* has a facilitating role in regulating locomotor activity in the open field. A limitation of our results is that the knockout mice were only backcrossed to C57BL/6N for six generations (Meng *et al*, 2008) and are not congenic. Thus, the effect of the mutation on locomotor activity could be an artifact caused by the mixed background. However, the first cohort of mice we tested included littermate controls, which addresses this concern by randomizing all unfixed alleles. This cohort showed results very similar to those in Figure 3 (data not shown), suggesting that the KO allele and not the mixed genetic background caused the observed phenotypic differences.

Our results do not demonstrate that a polymorphism in *Csnk1e* causes the QTL on chromosome 15. There are over 400 potentially functional polymorphisms in over 40 genes within the 79–82 Mb congenic region (Figure 2b and Supplementary Table S2). In principal, any of these polymorphisms could underlie the QTL. However, it is interesting to note that PF only enhanced MA sensitivity in B6.D2^{Csnk1e} mice and not B6.B6^{Csnk1e} mice (Figure 5a and b), consistent with the hypothesis that genetic variation in *Csnk1e* could be responsible for the differential effect of Csnk1e inhibition on MA-induced locomotor activity. This difference cannot be explained by insufficient dosing of PF because B6.B6^{Csnk1e} mice responded to PF-induced enhancement of fentanyl-induced locomotor activity (Figure 5c). Nor can it be explained by too high of a dose because PF did not affect MA sensitivity in B6.B6^{Csnk1e} mice using a lower dose either (20 mg/kg; data not shown). The result *could* be explained by genetic variation in *Csnk1e* that affects its expression and/or function. Alternatively, we may have been underpowered to detect the PF-induced enhancement in B6.B6^{Csnk1e} mice (Figure 5a), given that PF had a more subtle effect on MA-induced locomotor activity in B6.D2^{Csnk1e} mice (Figure 5b) compared with the large enhancement observed in both genotypes on fentanyl-induced locomotor activity (Figure 5c and d). Because of the rapid onset of fentanyl-induced locomotor activity, the effect of PF may have been easier to observe in both genotypes.

In contrast to the results observed with PF-4800567, we previously found that the CK-1 inhibitor PF-670462

decreased MA sensitivity (Bryant *et al*, 2009b; Li *et al*, 2011). PF-670462 was previously thought to be slightly more selective for Csnk1e over Csnk1d (Badura *et al*, 2007), but a recent study showed that PF-670462 was six-fold more selective for Csnk1d over Csnk1e and that PF-4800567 was 20-fold more selective for Csnk1e over Csnk1d (Walton *et al*, 2009). Because PF-4800567 increased MA and fentanyl sensitivity whereas PF-670462 decreased MA sensitivity, the combined results provide further evidence that Csnk1e and Csnk1d exert opposing regulatory roles on the locomotor stimulant response to drugs of abuse.

Psychostimulants and opioids induce a locomotor stimulant response via dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988). This leads to the activation of DARPP-32 in part via CK-1 (Greengard, 2001) and we previously showed that coadministration of the Csnk1d-preferring compound PF-4076462 inhibited MA-induced DARPP-32 phosphorylation (Bryant *et al*, 2009b; Li *et al*, 2011). As such, how is it that two closely related isoforms might exert opposing effects on sensitivity to drugs of abuse? One possibility is that cell type-specific expression of the two isoforms mediates the results. Although the general distribution of expression of Csnk1e and Csnk1d in the brain is similar, specificity of Csnk1e and Csnk1d expression has been observed in cell types such as Purkinje cells and astroglia (Utz *et al*, 2010; Lohler *et al*, 2009). Furthermore, a recent study showed that loss of DARPP-32 in D1-expressing cells reduced baseline and cocaine-induced locomotor activity whereas loss of DARPP-32 in D2-expressing cells increased baseline and cocaine-induced locomotor activity (Bateup *et al*, 2010). Thus, it is possible that Csnk1d is preferentially expressed in D1-containing cells and that Csnk1e is expressed in D2-containing cells. Interestingly, in examining the transcriptional profile of D1- *vs* D2-expressing cells following acute cocaine treatment, a significant increase in expression of Csnk1d was observed specifically in D1 cells and not D2 cells (Heiman *et al*, 2008). A separate possibility is that Csnk1e and Csnk1d modulate signaling pathways other than DARPP-32 that are responsible for their differential effects on drugs of abuse.

One unanswered question is the nature of the polymorphism in *Csnk1e* that we hypothesize to account for the observed QTL. There are several *Csnk1e* SNPs between the B6 and D2 strains, some of which are located near the transcriptional start site (http://www.informatics.jax.org), and thus could affect expression. The latest DNA sequencing results of inbred mouse strains (Keane *et al*, 2011) indicate that there are 12 potentially functional *Csnk1e* SNPs between B6 and D2 located within the 3′ UTR (Supplementary Table S2), any of which could influence mRNA stability. Additionally, *cis*-acting expression QTLs near *Csnk1e* derived from B6 and D2 genotypes have been observed in several brain areas using multiple microarray platforms (http://www.genenetwork.org). We and others did not observe a difference in Csnk1e expression in the striatum either via real-time quantitative PCR (data not shown) or via RNA sequencing (Bottomly *et al*, 2011). However, it is possible that a difference in *Csnk1e* expression at other developmental time points, other brain areas, or in specific cell types mediates the observed association with behavior.

The present series of studies combine forward and reverse genetics with pharmacological methods to identify *Csnk1e* as a negative genetic regulator of sensitivity to psychostimulants and opioids. Future studies will examine the brain areas, cell types, and signaling mechanisms by which we propose Csnk1e and Csnk1d to exert their dual regulatory roles in drug sensitivity. It will be important to examine the differential role that Csnk1e and Csnk1d might play in drug reward and reinforcement (Zhang *et al*, 2006) and the response to other abused substances such as alcohol.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)