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Inhibition of CaMKII in the Nucleus Accumbens Shell Decreases Enhanced Amphetamine Intake in Sensitized Rats

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Abstract

Microinjection of the calcium/calmodulin-dependent protein kinase II (CaMKII) inhibitor KN-93 into the nucleus accumbens (NAcc) shell impairs expression of the sensitized locomotion and NAcc dopamine (DA) overflow normally observed in psychostimulant-exposed rats. Based on these results, we investigated the effect of NAcc shell KN-93 on the enhanced amphetamine (AMPH) intake normally observed in AMPH- relative to saline-exposed rats. Rats were administered five injections of either AMPH (1.5 mg/kg, i.p.) or saline, one injection every two-three days. Fourteen days following the last injection, they were trained to self-administer AMPH (200 μg/kg/infusion, i.v.) first on fixed ratio schedules (FR) and then on a progressive ratio schedule of reinforcement (PR). As expected, AMPH-exposed rats worked harder and obtained significantly more drug infusions than saline-exposed rats on the PR schedule. After four days of stable responding, all rats were bilaterally microinjected with KN-93 (1 or 10 nmol/0.5 μl/side) into the NAcc shell, two minutes prior to the beginning of the self-administration session. Inhibiting CaMKII in this site reduced the enhanced drug intake observed in AMPH-exposed rats to levels no longer significantly different from those of saline-exposed rats. Responding in these latter controls was not affected by KN-93 nor did KN-93 affect responding in AMPH-exposed rats when it was infused into the NAcc core. Thus, in a manner similar to what has been reported for sensitized locomotion and NAcc DA overflow, these results suggest that inhibiting CaMKII in the NAcc shell attenuates the enhanced motivation to obtain a drug reinforcer that is normally displayed in AMPH-exposed rats.

Keywords

Nucleus accumbens; CaMKII; sensitization; amphetamine; self-administration; KN-93

Repeated exposure to psychostimulants such as amphetamine (AMPH) enhances the ability of these drugs to produce locomotor activation and nucleus accumbens (NAcc) dopamine (DA) overflow, and also leads to enhanced drug taking [5,10,12,25,26,27]. These enhanced responses, manifestations of behavioral and neurochemical sensitization, have been proposed to model the transition from casual drug use to drug abuse and addiction [21,28]. Consistent with this view, rats previously exposed to AMPH that work harder than saline-exposed controls

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to obtain the drug under a progressive ratio (PR) schedule of reinforcement also show enhanced NAcc DA overflow compared to control animals [27].

The serine/threonine kinase CaMKII (calcium/calmodulin-dependent protein kinase II) is required for the induction of LTP [9] and is known also to contribute importantly to the induction [8] and expression (4,6,16,17) of sensitization by psychostimulants. CaMKII is highly expressed in forebrain sites like the NAcc [3] and inhibiting its activity in this site blocks the expression of sensitization by these drugs. While the acute effect of AMPH on DA release is calcium (Ca^{2+})-independent, sensitized AMPH-induced NAcc DA overflow is dependent on Ca^{2+} and is blocked by reverse dialysis of the CaMKII inhibitor KN-93 into the NAcc shell [16]. Similarly, microinjecting KN-93 into this site attenuates sensitized locomotor responding to cocaine [17]. KN-93 has also been shown to block sensitized AMPH-induced DA release in synaptosomal [4] and striatal slice preparations [6] obtained from rats previously exposed to AMPH. In addition to presynaptically regulating AMPH-induced DA overflow in the NAcc and striatum, recent evidence suggests that CaMKII also postsynaptically mediates psychostimulant-induced upregulation of AMPA receptors in medium spiny neurons of the NAcc shell to facilitate the reinstatement of drug seeking [1].

Together, the above findings suggest that CaMKII may contribute to a number of neuroadaptations resulting from exposure to AMPH, including sensitized locomotion and NAcc DA overflow and the functional upregulation of NAcc AMPA receptors [24]. In light of the evidence that exposure to AMPH also enhances AMPH self-administration, it is conceivable that NAcc shell CaMKII contributes to this adaptation as well. The present experiments assessed this possibility by investigating whether inhibiting CaMKII in the NAcc shell alters the enhanced AMPH self-administration observed in sensitized rats.

Male Long-Evans rats (Harlan Teklad, Madison, WI) weighing 250–275 g upon arrival were used. They were individually housed with food and water available at all times in a 12-h light/12-h dark reverse cycle room. All experiments were conducted in accordance with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. All procedures were conducted according to an approved IACUC protocol.

Starting 3–5 days after arrival, animals in different groups were administered a total of five injections of AMPH (1.5 mg/kg, i.p.) or saline, one injection every 2–3 days. Similar drug exposure regimens have been shown to result in robust sensitization of AMPH-induced locomotion and NAcc DA overflow as well as enhanced AMPH self-administration [10,26, 27]. Two weeks after the last exposure injection, during which rats were surgically implanted with an intravenous (i.v.) catheter [24] and chronic bilateral guide cannulae aimed at the NAcc shell or core, AMPH self-administration training and testing was initiated. For all surgical procedures, rats were anesthetized with a mix of ketamine (100 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.). For intracranial implantation of cannulae, animals were placed in a stereotaxic apparatus with the incisor bar positioned 5.0 mm above the interaural line and the guide cannulae (22 gauge, Plastics One, Roanoke, VA) aimed at the NAcc shell (A/P, +3.4; L, ±0.8; DV, −7.5) or core (A/P, +3.4;.L,±1.5; DV, −7.5). Coordinates are in mm from bregma and skull [14]. Cannulae were angled at 10° to the vertical and positioned 1 mm above the final injection site. Obturators (28 gauge) were placed in the guide cannulae and rats were returned to their home cages. The i.v. catheters were implanted 5–7 days later using procedures generally described previously [18]. These were made of silastic tubing (Dow Corning, Inc), inserted into the right internal jugular vein, and positioned to exit slightly caudal of the midscapular region. Catheters were subsequently flushed daily with a sterile 0.9% saline solution containing 30 IU/ml heparin and 250 mg/ml ampicillin in order to promote patency.

Training for AMPH self-administration began 3 days after i.v. catheter implantation (14 days after the final drug exposure injection and 8–10 days after implantation of the intracranial guide cannulae). Ten test chambers (22 × 22 × 33 cm) containing a single retractable lever (5 cm above the floor) and a stimulus light positioned 13.5 cm above the lever were used. Each chamber was equipped with a counterbalanced arm, a steel-spring tether, and an infusion pump (model A.E., Razel Scientific Inc., Stamford, CT) that allowed free movement of the animal in the chamber and delivery of drug upon depression of the lever. Lever presses and drug infusions were recorded and controlled via an electrical interface by a computer using locally developed software. AMPH self-administration sessions were held daily and lasted for a maximum of 3 h. In all cases, reinforced lever presses delivered an infusion of AMPH through the i.v. catheter (200 μg/kg/infusion). An experimenter-delivered AMPH priming infusion was given at the beginning of each session and rats were required to self-administer 10 infusions of AMPH in a 3 hr session first on an FR1 and then an FR2 schedule of reinforcement. Animals that did not satisfy each of the FR1 and the FR2 criteria within 5 days were excluded from the study. Eight rats were thus excluded (AMPH-exposed, 2; saline-exposed, 6). Days to satisfaction of the training criteria under each FR schedule were recorded. Five additional rats were excluded due to nonpatent catheters or the development of leaks.

Upon satisfactory completion of training under the FR schedules, rats were tested daily under a PR schedule of reinforcement for 7 days. Under this schedule, the number of responses required to obtain each successive infusion of AMPH was determined by ROUND (5 × EXP (0.25 × infusion number) − 5) to produce the following sequence of required lever presses: 1, 3, 6, 9, 12, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208, etc [20]. The daily PR sessions were terminated after 3 hr or after 1 hr elapsed without a drug infusion. Priming AMPH infusions were not given on these sessions. On the first 4 sessions, rats received no NAcc infusion prior to self-administration to establish enhanced AMPH self-administration in sensitized rats. On the fifth PR session, rats were administered a bilateral microinjection of KN-93 into the NAcc shell (1 or 10 nmol/0.5 μl/side) or, for control, the NAcc core (10nmol/0.5μl/side) over a period of 1 minute with an additional minute to allow for diffusion. Doses were chosen based on previous reports showing that 10 nmol/0.5μl/side KN-93 did not affect acute cocaine-induced locomotion but selectively blocked the expression of sensitized locomotor responding to cocaine [17]. Immediately after the microinjection, animals were allowed to self-administer AMPH under the PR schedule. Finally, rats were tested on the remaining two sessions again with no NAcc infusion prior to self-administration. The number of infusions obtained in each PR session was recorded. S(+)-amphetamine sulfate (AMPH) was obtained from Sigma Inc. (St. Louis, MO) and was dissolved in sterile 0.9% saline. KN-93 was obtained from Calbiochem (San Diego, CA) and was dissolved in water.

At the end of the experiments, rats were anesthetized with sodium pentobarbital and perfused with saline and 10% formalin. Coronal sections (40 μm) were mounted on gelatin-coated slides and stained with cresyl violet for verification of cannulae tip placements. Only subjects with both cannulae in the NAcc shell or in the NAcc core were included in the analyses (Fig. 1). Eighteen rats were excluded for failing to meet this criterion (AMPH-exposed, 10; Saline-exposed, 8). As reported by others [17], cannula tip placements in both the ventral and medial limbs of the shell were included. There was no evidence to indicate that infusion into these two sites within the NAcc shell produced differential effects. Statistical analyses were performed according to Kirk [7].

Consistent with previous reports [10,27], rats previously exposed to AMPH (n=9) or saline (n=8) similarly satisfied each of the FR1 and FR2 self-administration training criteria within 1–2 days. No significant group differences in days to criterion were detected with independent samples t-tests for either ratio (t_{15}=−0.16–1.38, NS). Again, consistent with previous reports [10,27], rats previously exposed to AMPH worked more and obtained significantly more

AMPH infusions under the PR schedule of reinforcement compared to saline-exposed controls. This effect was maintained over the first 4 PR days of testing (Fig. 2). When number of infusions obtained was analyzed with a between-within ANOVA with exposure (AMPH, saline) as the between factor and days of testing (4) as the within factor, a significant effect of exposure was detected ($F_{1,15}$=8.76, P<0.01). This enhanced work output and self-administration of AMPH in rats previously exposed to the drug was significantly reduced when KN-93 (10nmol/0.5μl/side) was infused into the NAcc shell prior to self-administration on the following PR test day. This infusion of KN-93 was without effect in saline-exposed controls (Fig. 3). Comparing number of infusions obtained on PR test day 4 to those obtained on the following KN-93 test day, a between-within ANOVA with exposure (AMPH, saline) as the between factor and test days (PR4, KN-93) as the within factor detected a significant effect of test days ($F_{1,15}$=10.72, P<0.01) and a significant exposure by test days interaction ($F_{1,15}$=7.58, P<0.05). Post-hoc Scheffé comparisons revealed that AMPH-exposed rats obtained significantly fewer AMPH infusions following NAcc shell KN-93 than on PR test day 4 (P<0.001). In addition, while AMPH-exposed rats obtained significantly more AMPH infusions than saline-exposed rats on PR test day 4 (P<0.05), a significant difference between groups was no longer detected on the KN-93 test day. In AMPH-exposed rats, responding returned to pre-test levels following the KN-93 test day. The number of infusions obtained on the two subsequent sessions (8.44±1.73 and 9.67±1.83) did not differ significantly from those observed on PR test day 4 (t_8=1.71 and 0.51, NS). In saline-exposed rats, responding did not differ significantly from pre-test levels in these two sessions (5.50±1.51 and 7.5±1.66; t_7=0.98 and 1.66, NS).

When a lower dose of KN-93 (1 nmol/0.5μl/side) was microinjected into the NAcc shell of separate AMPH-exposed rats, the enhanced self-administration observed in these animals was not significantly affected (Fig. 3 inset). Similarly, microinjection of 10nmol/side KN-93 into the NAcc core of separate AMPH-exposed rats was without effect (Fig. 3 inset), suggesting that CaMKII is acting in a site-specific manner to regulate enhanced drug self-administration. When number of AMPH infusions obtained by these two groups on the KN-93 test day was compared to that of AMPH-exposed rats administered 10 nmol/side KN-93 into the NAcc shell, only the latter group showed a significant decrease relative to PR test day 4. A between-within ANOVA with groups as the between factor (3) and test days (PR 4, KN-93) as the within factor detected a significant groups by test days interaction ($F_{2,18}$=4.91, P<0.05). Post-hoc Scheffé comparisons revealed a significant decrease in AMPH infusions obtained from PR test day 4 to the KN-93 test day only in rats administered 10 nmol/side KN-93 into the NAcc shell (P<0.001).

The present results show that inhibiting CaMKII in the NAcc shell decreases the enhanced work output and AMPH self-administration normally observed in rats previously exposed to the drug. This finding is consistent with those of previous reports showing that CaMKII contributes to a number of pre-and postsynaptic neuroadaptations resulting from exposure to AMPH, including sensitized NAcc DA overflow [16] and the functional upregulation of NAcc AMPA receptors [24]. Both of these neuroadaptations have been linked to enhanced drug self-administration and reinstatement [1,10,24,27], suggesting that the latter are behavioral manifestations of CaMKII-dependent neuronal changes initiated by exposure to AMPH.

AMPH has been reported to produce sensitized locomotion and DA overflow preferentially in the NAcc shell [15]. The present results showing that KN-93 was effective in the shell but not the core of the NAcc are consistent with this finding and can be interpreted to suggest that CaMKII influences the generation of sensitized behavior in this site by regulating DA overflow. CaMKII activity has been shown to enhance AMPH-induced DA efflux both in vitro and in vivo by directly interacting with the DA transporter [2]. And, indeed, sensitized NAcc DA

overflow accompanies enhanced AMPH self-administration [27] and manipulations that prevent induction of the former equally prevent induction of the latter [19,22,23].

It is important, however, to also consider postsynaptic sites of action of CaMKII in enhanced AMPH self-administration. Previous exposure to AMPH functionally upregulates NAcc AMPA receptors as evidenced by enhanced NAcc AMPA induced reinstatement compared to that observed in saline-exposed controls [24]. Evidence suggests that psychostimulant-induced upregulation of AMPA receptors in NAcc medium spiny neurons is dependent on CaMKII [1]. Interestingly, this was reported to be the case in NAcc shell but not core. Preliminary findings have also shown that viral-mediated overexpression of αCaMKII, again in NAcc shell, leads to enhanced locomotor responding to AMPH in drug-naïve rats [11]. Thus, the present findings with NAcc shell KN-93 are also consistent with the possibility that CaMKII acts postsynaptically in this site to mediate enhanced AMPH self-administration. Recently, it was shown that cocaine reinstatement is dependent on D1 DA receptor-dependent increases in CaMKII activity (autophosphorylation at Thr286) and phosphorylation of the AMPA receptor subunit GluR1 at ser831 (a known CaMKII residue) in NAcc shell, leading to the intriguing possibility that CaMKII may provide a biochemical bridge linking NAcc DA and glutamate transmission in this site [1]. Whatever the case, the present findings clearly demonstrate an important role for NAcc shell CaMKII in mediation of enhanced AMPH self-administration and identify it as a potential target for therapeutic intervention in the treatment of psychostimulant addiction.

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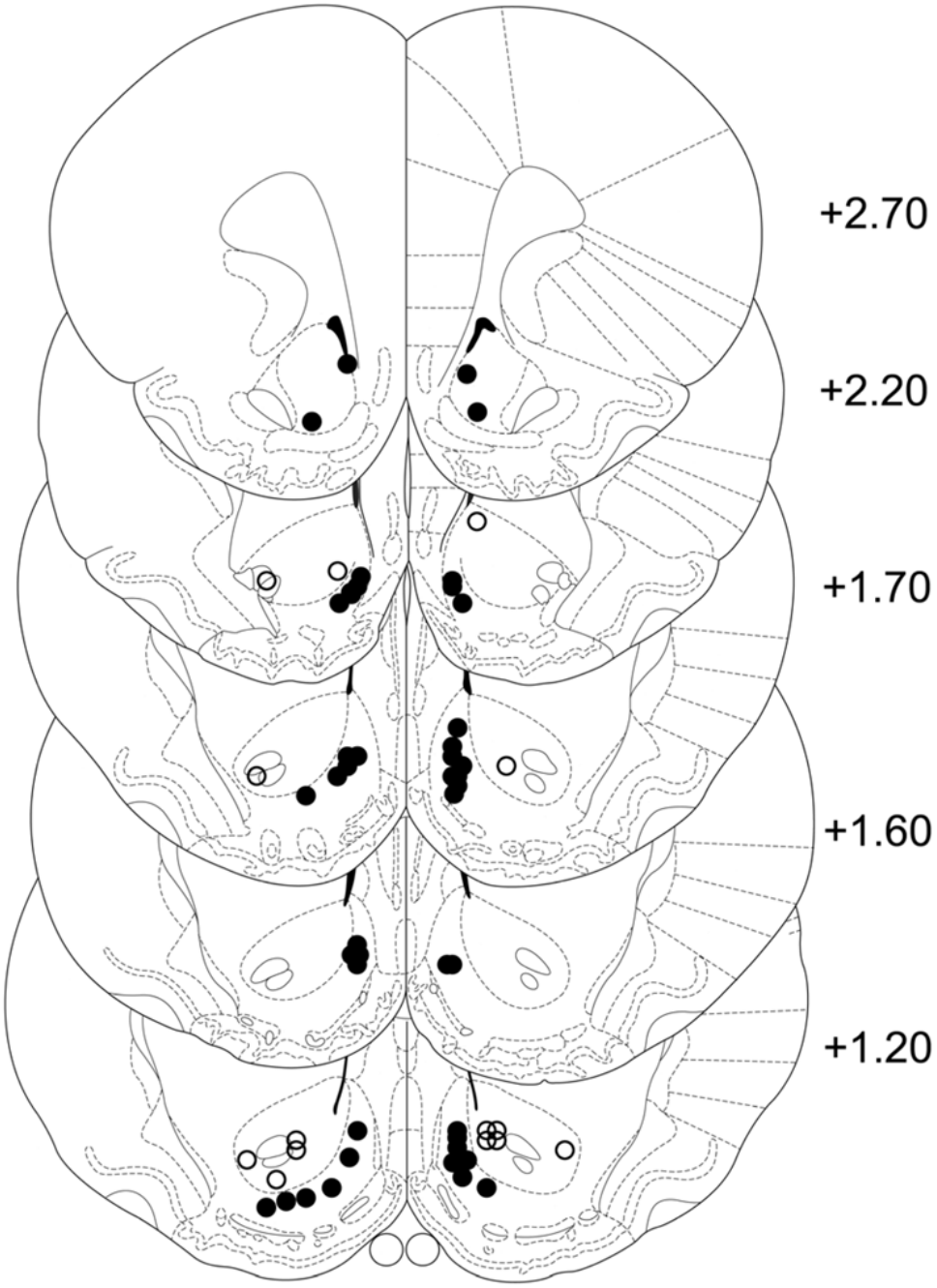

Figure 1. Injection cannula tip placements in the NAcc shell and core

Black circles represent placements in the NAcc shell; open circles represent placements in the NAcc core. Line drawings are from Paxinos and Watson [13]. Numbers to the right indicate mm from bregma.

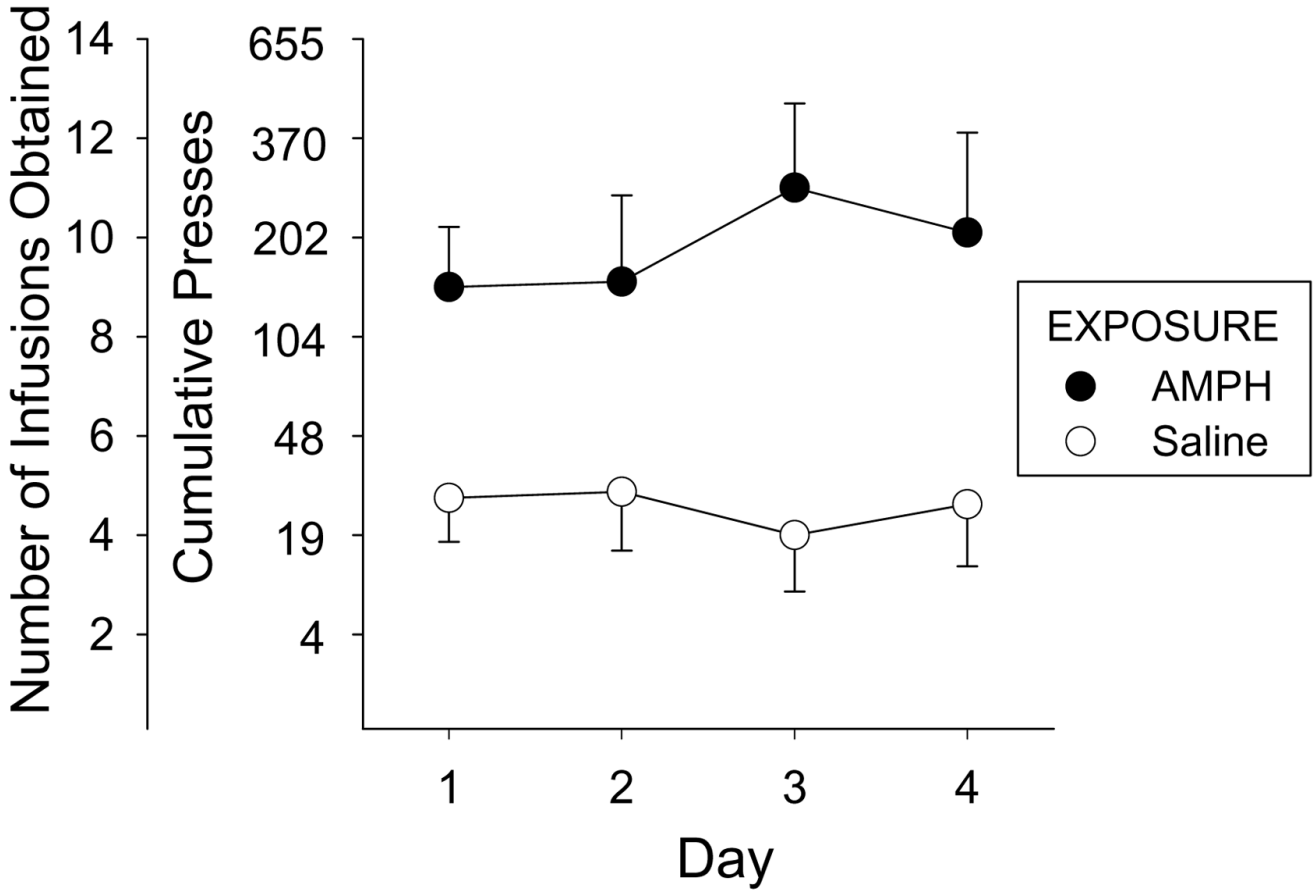

Figure 2. Previous exposure to AMPH enhances AMPH self-administration under a PR schedule of reinforcement

Data are shown as mean (±SEM) number of AMPH infusions obtained on each of the first 4 PR test days. The cumulative number of presses required to obtain these infusions is also shown. AMPH-exposed rats worked more and consequently obtained significantly more infusions compared to saline-exposed controls over all 4 PR test days. n=8–9/group.

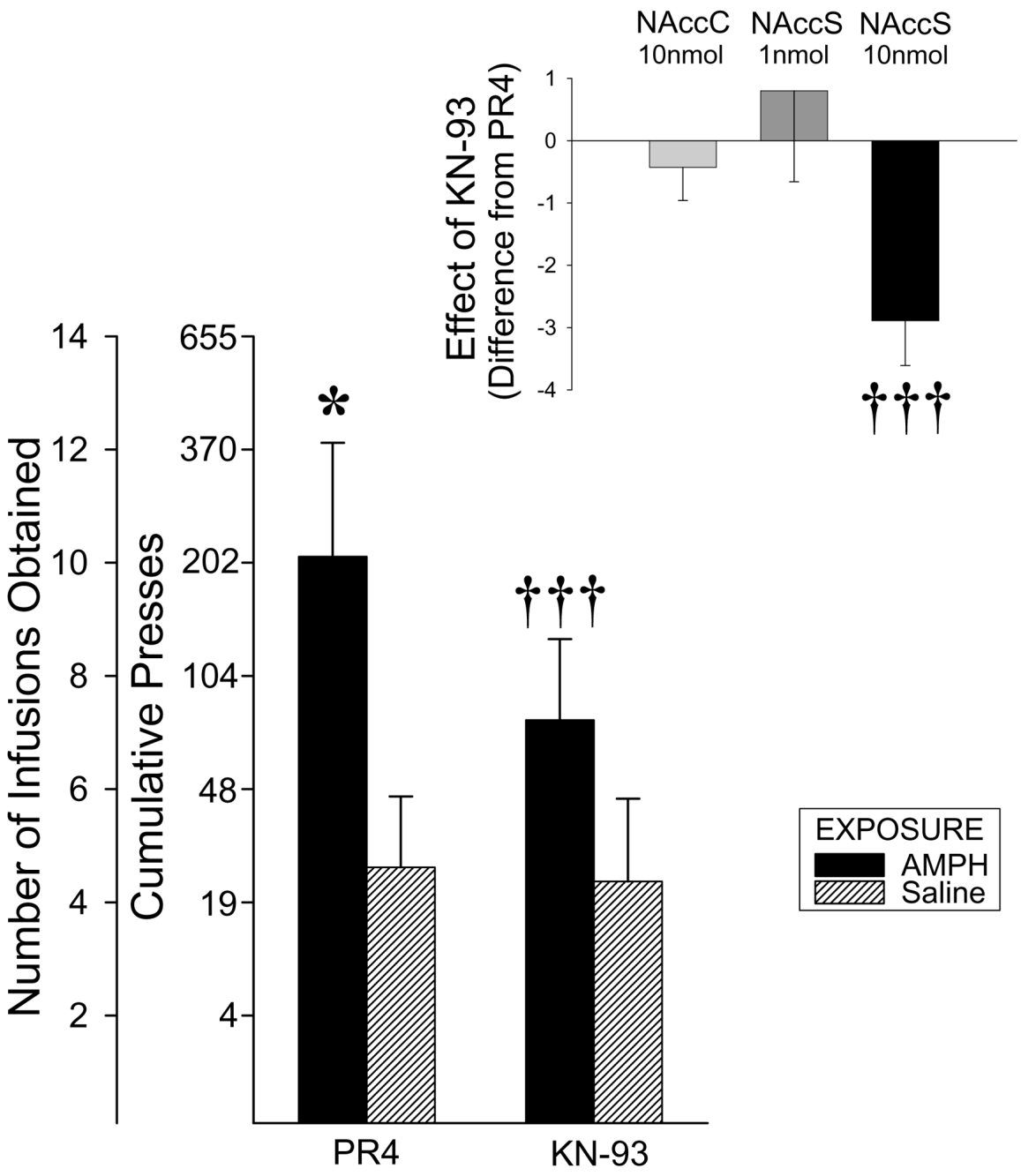

Figure 3. Microinjection of KN-93 into the NAcc shell reduces the enhanced AMPH self-administration observed in sensitized rats

Data are shown as mean (+SEM) number of AMPH infusions obtained on PR test day 4 (PR4) and the 10 nmol/side KN-93 test day (KN-93). The cumulative number of presses required to obtain these infusions is also shown. Following the KN-93 microinjection, AMPH-exposed rats showed a significant reduction in drug intake compared to that observed on PR test day 4. Inhibiting CaMKII produced no effect in saline-exposed controls. n=8–9/group. *, P<0.05, significantly different from saline-exposed controls at PR test day 4. †††, P<0.001, significantly different from PR test day 4. **INSET: Microinjection of 1.0 nmol/side KN-93 into the NAcc shell or 10 nmol/side KN-93 into the NAcc core had no effect on drug intake in AMPH-**

exposed rats. Data are shown as the difference in the number of infusions obtained on the KN-93 test day from that obtained on PR test day 4 in AMPH-exposed rats tested with KN-93 in the NAcc core (10 nmol/side; n=7), NAcc shell (1.0 nmol/side; n=5), and NAcc shell (10 nmol/side; n=9). †††, $P<0.001$, significantly different from PR test day 4.