

## REGULAR RESEARCH ARTICLE

# Task-Dependent Effects of SKF83959 on Operant Behaviors Associated With Distinct Changes of CaMKII Signaling in Striatal Subareas

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## Abstract

**Background:** SKF83959, an atypical dopamine (DA) D1 receptor agonist, has been used to test the functions of DA-related receptor complexes in vitro, but little is known about its impact on conditioned behavior. The present study examined the effects of SKF83959 on operant behaviors and assayed the neurochemical mechanisms involved.

**Methods:** Male rats were trained and maintained on either a fixed-interval 30-second (FI30) schedule or a differential reinforcement of low-rate response 10-second (DRL10) schedule of reinforcement. After drug treatment tests, western blotting assayed the protein expressions of the calcium-/calmodulin-dependent protein kinase II (CaMKII) and the transcription factor cyclic AMP response element binding protein (CREB) in tissues collected from 4 selected DA-related areas.

**Results:** SKF83959 disrupted the performance of FI30 and DRL10 behaviors in a dose-dependent manner by reducing the total number of responses in varying magnitudes. Moreover, the distinct profiles of the behavior altered by the drug were manifested by analyzing qualitative and quantitative measures on both tasks. Western-blot results showed that phospho-CaMKII levels decreased in the nucleus accumbens and the dorsal striatum of the drug-treated FI30 and DRL10 subjects, respectively, compared with their vehicle controls. The phospho-CREB levels decreased in the nucleus accumbens and the hippocampus of drug-treated FI30 subjects but increased in the nucleus accumbens of drug-treated DRL10 subjects.

**Conclusions:** Our results provide important insight into the neuropsychopharmacology of SKF83959, indicating that the drug-altered operant behavior is task dependent and related to regional-dependent changes of CaMKII-CREB signaling in the mesocorticolimbic DA systems.

**Keywords:** Atypical dopamine receptor agonist, dorsal striatum, neurochemical mechanism, nucleus accumbens, schedule-controlled behavior

## Introduction

Dopamine (DA) transmission plays a critical role in motivation and relevant behavioral processes (Phillips et al., 2008; Haber and Knutson, 2010; Salamone and Correa, 2012; Lammel et al., 2014; Bamford et al., 2018). While the perturbation of DA neurotransmission is implicated in the development of psychiatric

disorders (Salamone et al., 2015; Grace 2016), the underlying neural mechanisms remain to be elucidated. To specifically probe the dopaminergic involvement in certain features of motivated behavior, the operant behavioral paradigms provide well-adopted animal models to determine the effects of

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## Significance Statement

We examined the effects of SKF83959, an atypical dopamine receptor agonist, on operant behaviors and the protein expressions of CaMKII and CREB in male rats. Acute SKF83959 dose-dependently reduced the response-related measures of the FI30 and DRL10 tasks in varying magnitudes. The task-dependent effects were further explored through the qualitative and quantitative analyses of inter-response-time data. In contrast to its effects on operant behavior, SKF83959 did not affect spontaneous locomotor activity, thereby indicating the presence of different neurobiological substrates underlying locomotion and lever pressing. The examination of protein phosphorylation changes due to SKF83959 showed task- and brain region-dependent patterns. The levels of phospho-CaMKII and phospho-CREB in the dorsal striatum and nucleus accumbens were correlated with either or both of the operant behaviors altered by the drug. These results elucidated the behavioral and neurochemical effects of SKF83959, which may contribute to the development of dopaminergic drugs for motivational/cognitive control-related disorders.

drug treatment in preclinical research (Sanger and Blackman, 1989; van Haaren, 1993). Here, we focused on 2 timing-relevant operant behaviors, including a fixed-interval (FI) schedule and a differential reinforcement of low-rate response (DRL) schedule. Despite the common timing process, operant behavior performed on FI task was distinctively different from that performed on DRL task (Ferster and Skinner, 1957). FI behavior displays a higher response rate than DRL behavior because the latter requires behavioral inhibition. As suggested by the principle of response rate dependency in behavioral pharmacology (Dews, 1955), the effectiveness of a particular drug on behavior could be dependent on the baseline response rate in the absence of that drug. In the present study, we applied these 2 operant behavior tasks to evaluate SKF83959, an atypical DA receptor agonist.

SKF83959, a phenylbenzazepine derivative, has been shown to act as a partial agonist on D1-like DA receptors (Neumeier et al., 2003). Behavioral characteristics of D1 receptor activation, such as grooming, vertical jaw movements, incisor chattering, and contralateral rotations, were induced by SKF83959 in rats with unilateral 6-hydroxydopamine lesions (Downes and Waddington, 1993; Deveney and Waddington, 1995; Gnanalingham et al., 1995; Panchalingam and Undie, 2001; Makiyama et al., 2007). However, biochemically, SKF83959 exhibited antagonist-like effects on DA receptors via the failure to stimulate adenylyl cyclase activity and inhibition of DA-induced adenylyl cyclase activity (Arnt et al., 1992). Panchalingam and Undie (2001) reported an induction of phospholipase C-mediated phosphoinositide (PI) hydrolysis in rat and monkey brain slices on SKF83959 treatments; hence, SKF83959 could act on the PI-linked D1-like receptors (Undie and Friedman, 1990; Undie et al., 1994). More recently, the PI-linked signaling unit was hypothesized to be composed of heterodimerized D1-D2 receptors, which can be activated by SKF83959 to stimulate  $G_{q/11}$  proteins with phospholipase C-mediated PI hydrolysis and then the release of intracellular calcium followed by the activation of the calcium-/calmodulin-dependent protein kinase II (CaMKII) (George and O'Dowd, 2007; Rashid et al., 2007; Hasbi et al., 2010; but see Chun et al., 2013). Along with research that probes the neurochemical actions of SKF83959, a number of studies examined the effects of this compound on rat behavior, including acoustic startle reflex (Zhang et al., 2005), eye blinking (Desai et al., 2007), maternal behavior (Stolzenberg et al., 2010), locomotor activity (Cools et al., 2002; Zhang et al., 2007; Perreault et al., 2010), stimulant-induced locomotor sensitization (Shen et al., 2015b; Hasbi et al., 2018), anxiety-like response (Shen et al., 2015a; Hasbi et al., 2020a), and DA drug-induced dyskinesia (Zhang et al., 2007). The behavioral data of SKF83959 were also reported in primates (e.g., Platt et al., 2000). These abovementioned studies used the unconditioned behavioral models, but the behavioral effects of SKF83959 on the instrumental/operant conditioning paradigm have rarely been examined. Accordingly, this study was designed to investigate

the effects of SKF83959 on FI and DRL schedule-controlled behaviors (Experiment 1). Considering the potential involvement of the D1-D2 receptor heteromer signaling linked with drug-altered operant behaviors, the expressions of the CaMKII and the transcription factor cyclic AMP response element binding protein (CREB) were analyzed by western blot to examine the neurochemical effects of SKF83959 treatment (Experiment 2). The brain regions of interest were the prefrontal cortex (PFC), dorsal striatum (DS), nucleus accumbens (NAc), and dorsal hippocampus (Hippo). A separate group of rats was used to test the dose effects of SKF83959 on spontaneous locomotor activity (Experiment 3) to verify whether the manifestation of drug effect depended on different behavioral paradigms (i.e., locomotion vs operant).

## Methods

### Subjects

Male Wistar rats (BioLASCO Taiwan Co., Ltd) weighing approximately 250 g on receipt were the subjects. The rats were housed in a temperature-controlled colony ( $23 \pm 1^\circ\text{C}$ ) under a 12-h-light/-dark cycle (light on at 7:30 AM). After 10 days of acclimatization to the food and water provided ad libitum and being handled daily, the rats for operant training and test were maintained on a water-restriction regimen. That is, the rats had 5–15 minutes of access to tap water in the home cage 30 minutes after the end of each daily experimental session. Food pellets were always available in each home cage. A water-restriction regimen was also applied to the rats used in the locomotor activity test. All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the institutional committee of animal use and care of National Cheng-Chi University.

### Apparatus

Operant behavioral measures were conducted using a custom-made operant system with 4 chambers. The interior dimensions of each chamber were  $20 \times 25 \times 30 \text{ cm}^3$  (Med Associates, St. Albans, VT, USA). The interior design of the operant chamber and its equipped parts were the same as previously reported (e.g., Liao and Cheng, 2005; Cheng et al., 2006). The 4 operant chambers were serviced and controlled by a microcomputer with an in-house-designed program to control the operant environment and to allow data collection.

A locomotor activity test was conducted in an assembly of 4 identical black acrylic boxes ( $45 \times 45 \times 36 \text{ cm}^3$  each). A charge-coupled device camera was installed above the center of the assembly at a height of 52 cm from the ground. The camera was connected to a desktop computer in which the

distance travelled by the rats was recorded using a commercial software (SINGA Real-Time Trace System, Taiwan).

## Drugs

SKF83959 hydrobromide (Tocris Bioscience) was dissolved in the vehicle containing 10% ethanol. The drug was i.p. injected at a volume of 1 mL/kg of body weight. Drug administration was conducted 30 minutes before the start of the behavioral session. The doses and pretreatment time chosen were referred to previous studies (Zhang et al., 2005; Desai et al., 2007).

## Training of FI30 and DRL10 Behaviors

The water-deprived rats were subjected to magazine training, during which they learned to associate water with the metal receiving dish. Then, the rats were run for 3 daily sessions (30 minutes each) of shaping by pressing a lever to acquire the water reinforcer based on the fixed-ratio 1 schedule of reinforcement. All rats were able to make at least 65 lever presses in a 30-minute training session, thereby meeting the criterion of this stage. The rats were then randomly assigned into separate groups for training in either the FI (n=12) or DRL (n=12) task.

In the FI task, the reinforcer was contingent to the first lever press made after the time interval since the previous reinforcer. Lever presses during the time interval had no reinforcement contingencies. Rats in the FI group began daily training under 1-hour sessions of the FI 10-second schedule for 10 days before shifting to daily 30-minute training sessions under FI 30-second schedule (FI30) for 25 days. The average number of total responses reached 600 per day in the last 3 days of FI30 training, which was the criterion of baseline performance before the commencement of the pharmacological test sessions. In the DRL task, the rats had to wait a specified interval in seconds between lever presses to obtain the reinforcer. Any response made before the criterion time would reset the DRL clock, and no reinforcer was obtained. The rats in the DRL group were trained by hourly sessions of the DRL 10-seconds (DRL10) schedule for 10 days followed by daily 30-minute training sessions for 25 days. The testing of drug-induced performance began when the average number of total responses reached 200 per day in the last 3 days of training.

## Procedures

In Experiment 1, the dose effects of SKF 83959 on the behavioral performance of FI30 and DRL10 schedules were evaluated when the rats acquired a stable baseline performance in the operant tasks. One rat in the DRL10 group did not meet the baseline criterion and was excluded from the drug test. The treatments of SKF83959 at 0, 0.01, 0.1, and 1.0 mg/kg were applied in a within-subject design in the FI30 (n=12) and DRL10 (n=11) groups. The dose order was counterbalanced using a Latin square design tested for 4 days consecutively. The duration of the behavioral test was 30 minutes for each drug or vehicle treatment.

Experiment 2 was conducted to investigate the behavioral and neurochemical effects of SKF83959. The rats from Experiment 1 underwent 6 days of retraining on respective tasks to return to the stable baseline. The rats in the FI30 and DRL10 groups were then divided to receive either the vehicle (n=6 in the FI30 group and n=5 in the DRL10 group) or the 1-mg/kg dose of SKF83959 (n=6 for each for FI30 and DRL10) in a between-subject design. The rats in the FI30 group that made less than 3 lever presses in Experiment 1 (#15, 17, 20, 21, and 23; [supplementary Table 1](#)) were randomly distributed to the vehicle group (#17, 21, and

23) and the drug treatment group (#15 and 20). The rat in the DRL10 group that made no lever presses in Experiment 1 (#3; [supplementary Table 1](#)) was placed into the vehicle group in Experiment 2. In the subject assignment, no significant differences were found in the baseline of total responses between groups before the drug injections in Experiment 2. The vehicle group made 562±98 responses, and the experimental group made 565±53 responses in the FI30 schedule ( $F=0.0008$ ,  $P>.05$ ). And the vehicle group made 195±9 responses while the experimental group made 193±21 responses in the DRL10 schedule ( $F=0.0099$ ,  $P>.05$ ). The procedures of the drug test on operant behaviors were the same as those described in Experiment 1. After the behavior test, the rats were decapitated to remove the brain, which was dissected on an ice-cold plate. The tissues of the PFC, NAc, DS, and Hippo were collected using a micro-punch, treated with liquid nitrogen, and stored in a -80°C freezer until used for western-blot analysis (see below).

In Experiment 3, a separate batch of naive rats following the adaptation of the water-restriction regimen was divided into 3 groups receiving 0, 0.5, or 1.0 mg/kg of i.p. SKF83959 injection (n=5 each) to evaluate the effects of SKF83959 on spontaneous locomotor activity. After the respective drug injection, rats were placed in their holding cages in the behavioral test room for 30 minutes and then placed into open field boxes for the 30-minute locomotor activity test. The total travel distance was recorded in 5-minute intervals.

## Western Blotting

The collected brain tissues were homogenized with a radioimmunoprecipitation buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA), and 1% octylphenoxy poly(ethyleneoxy)ethanol (IGEPAL CA-630)) with Protease Inhibitor Cocktail Set I and the Protein Phosphatase Inhibitor Cocktail Set IV (Calbiochem). Equal amounts of lysate samples (20 µg protein) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membranes (Millipore). The polyvinylidene difluoride membranes were incubated with 2% bovine serum albumin (BSA) in Tris-buffer solution with 0.05% Tween 20 and then with 1 of the following primary antibodies: anti-phospho-CaMKII (Upstate), anti-CaMKII (Millipore), anti-phospho-ser133-CREB (Millipore), anti-CREB (Millipore), or anti-β-actin (Millipore). Horseradish peroxidase-conjugated secondary antibody (Perkin Elmer) was used for all western-blot assays. The membrane was detected with chemiluminescent horseradish peroxidase substrate (Millipore) to visualize the protein bands, which were quantified by NIH Image J software.

## Statistical Analysis

In the DRL10 behavioral measurement, each lever press was classified in terms of its associated inter-response time (IRT; the time in milliseconds elapsed since the prior response). The resulting dataset on IRT was grouped and plotted into a distribution that consisted of response frequencies for 21 consecutive 1-second time bins. Six dependent variables were studied for quantitative analyses, as follows: (1) number of total responses, (2) number of reinforced responses, (3) number of nonreinforced responses, (4) number of burst responses, (5) peak rate, and (6) peak time. The first 3 measures were simple frequency measures of the response maintained by the DRL procedure that can only report more or less response in rats under different dose treatments. The burst responses were summed frequencies of

IRTs that were <2 seconds (bins 1 and 2, as shown in IRT distribution curves of figures). The peak time and peak rate were calculated from de-burst IRTs (IRT >2 sec), in which a moving average based on 4 consecutive 1-second bins with 1-second step size was applied to smoothen the distribution. With the maximum frequencies of a 4-second epoch identified, the peak time was the average value (in milliseconds) of all IRTs that fell within those 4 bins (i.e., the maximal epoch). The measure of peak time indicates the time point at which the rats pressed the lever with the highest IRT frequency, thus reflecting the rats' expected criterion time of the reward in the DRL procedure. The peak rate was calculated from the summed response frequency in those 4 bins divided by 4, thus rendering a unit of responses per sec for peak rate. The smoothing procedure can prevent an abrupt frequency response change in the IRT distribution, especially when the frequency of response is low due to the nature of the DRL procedure; and this smoothing procedure has been used previously (e.g., Chiang et al., 2015).

In the FI30 behavioral measurement, the total number of responses and the number of reinforcers were analyzed in addition to the IRT distribution within a 30-second interval. The post-reinforcement pause (PRP) represented the duration of the first response made within a 30-second interval starting from the time point of the preceding reinforcement. The FI schedule PRP refers to the duration of time lapse from receiving a reinforcer to making the next lever press in the following interval. The PRP was chosen over other measures, such as the quarter-life and index of curvature, based on the following considerations: (1) a highly positive correlation being found among these 3 measures (Gollub, 1964; Dukich and Lee, 1973), and (2) the PRP represented a better measure for reflecting the subject's capability of behavioral inhibition under FI schedule (Staddon, 1969), since the inhibition component is essential in performing the DRL behavior.

Few rats appeared to be low-responsive or balking their operant response on the administration of 1.0 mg/kg SKF 83959 (see supplementary Table 1). Cases of such low responses (<5 total responses) were still included in the behavior analysis, because the exclusion of these cases in notable numbers may introduce bias to the results. Hence, all of the actual numbers were used in analyzing response-based indexes, but the group mean was substituted in the cases of missing data in other indexes as follows: PRP in the FI30 task, and peak time and peak rate in the DRL10 task.

The data are presented in mean  $\pm$  SEM and were analyzed with ANOVA using Statistica (version 7.1, StatSoft). Post-hoc comparisons were conducted using Tukey's honestly significant difference test with a significance level of  $P < .05$ . Bivariate correlations between behavioral measures and protein results were conducted using SPSS (version 16.0, SPSS Inc.).

## Results

### Experiment 1

The dose effects of SKF83959 on FI30 behavior are illustrated in Figure 1. The FI30 rats exhibited a dose-dependent decrease in response rates on the 30-second interval IRT curve as they received higher doses of SKF83959 injections (Figure 1A). One-way ANOVA showed that drug dose had significant effects on the numbers of total responses [ $F(3,33)=32.95$ ,  $P < .001$ , reinforced responses,  $F(3,33)=22.51$ ,  $P < .001$ ] and the duration of PRP [ $F(3,33)=26.11$ ,  $P < .001$ ]. As indicated by post-hoc test, the rats emitted significantly fewer numbers of total responses when they were injected with SKF83959 at the medium dose (0.1 mg/kg;  $P < .01$ ) and high dose (1.0 mg/kg;  $P < .001$ ) (Figure 1B). The rats

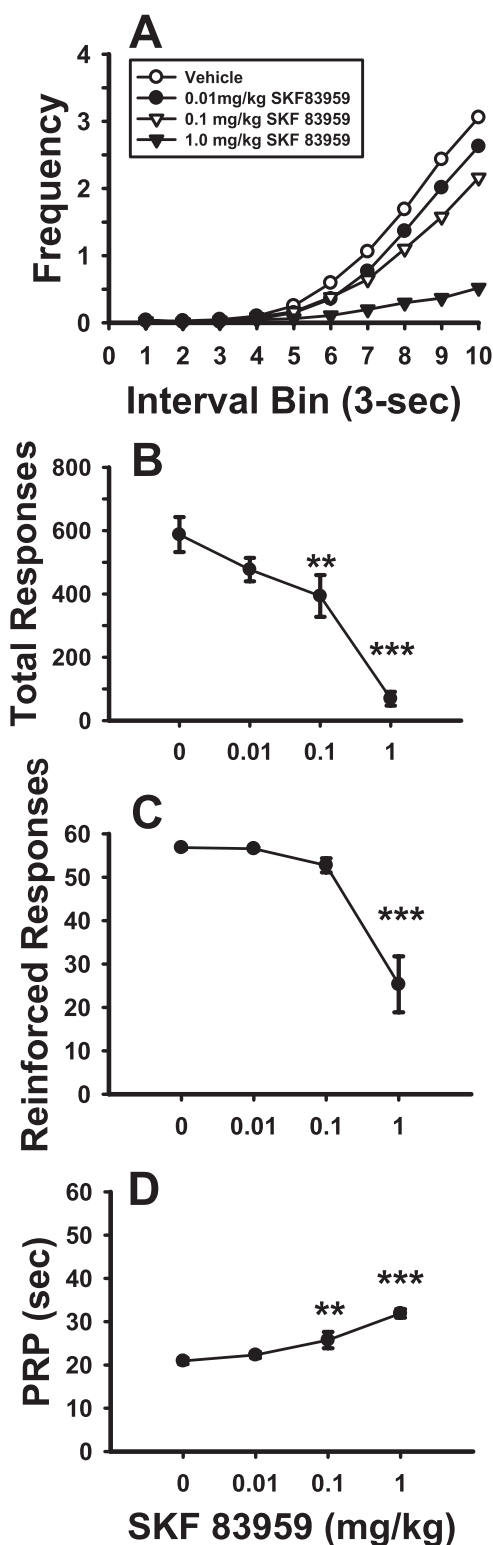


Figure 1. Dose effects of SKF83959 on fixed-interval 30-second schedule (FI30) behavior (Experiment 1) as measured by: inter-response time (IRT) distribution with the 30-second interval (A), total number of responses (B), the number of reinforced responses (C), and the duration of post-reinforcement pause (PRP). Asterisks denote significant differences between the indicated dose treatment and the vehicle control by post hoc tests: \*\* $P < .01$ , \*\*\* $P < .001$ .

also showed significantly fewer numbers of reinforced responses on high-dose SKF83959 administration (1.0 mg/kg;  $P < .001$ ; Figure 1C); their durations of PRP were significantly increased

after injections with medium- and high-dose SKF83959 ( $P < .01$  and  $P < .001$ , respectively) (Figure 1D). As noted in [supplementary Table 1](#), 3 of the 12 rats tested had completely ceased to respond on the lever during the test session on 1.0-mg/kg SKF83959 administrations, while an additional number of 2 rats emitted  $\leq 2$  lever presses during the session after high-dose injection.

The rats trained under DRL10 similarly showed a dose-dependent decrease in response rates on the IRT curve with higher doses of SKF83959 injections, while the peak time remained relatively in place (Figure 2A). One-way ANOVA found the drug dose to have significant effects on 3 of the 4 response-based indexes, including the numbers of total responses [ $F(3,30) = 4.53$ ,  $P < .01$ ] (Figure 2B), reinforced responses [ $F(3,30) = 3.49$ ,  $P < .05$ ] (Figure 2C), and non-reinforced responses [ $F(3,30) = 3.49$ ,  $P < .05$ ] (Figure 2D). Post-hoc tests revealed that the total responses, reinforced responses, and non-reinforced responses significantly decreased when the rats were treated with high-dose SKF83959 (all  $P < .05$ ). The number of burst responses was not affected by SKF83959 ( $P > .05$ ) (Figure 2E). The peak rate was significantly affected by the doses of SKF83959 [ $F(3,30) = 6.58$ ,  $P < .01$ ] (Figure 2F). Post-hoc test showed a significant decrease of peak rate under high-dose SKF83959 injection ( $P < .01$ ). The peak time was unaffected by SKF83959 administrations (Figure 2G). During the DRL test, 1 of the 11 rats emitted no responses on high-dose SKF83959 injection ([supplementary Table 1](#)).

## Experiment 2

The results of Experiment 2 are presented in 2 parts: behavioral pharmacology and western-blot assay. [Table 1](#) shows the effects of 1 mg/kg SKF83959 on FI30 and DRL10 behavior, which replicated the findings of Experiment 1 to a greater extent. Compared with the vehicle group of FI30, the SKF83959-treated rats had a significant decrease of total responses, [ $F(1,10) = 23.49$ ,  $P < .001$ ] and reinforced responses [ $F(1,10) = 16.12$ ,  $P < .01$ ]. The drug-treated rats exhibited significantly increased durations of PRP [ $F(1, 10) = 18.53$ ,  $P < .01$ ]. One of the 6 rats in the drug treatment group emitted no responses on SKF83959 injection. Regarding the DRL10 task, the drug-treated rats showed significantly decreased total responses [ $F(1,9) = 7.51$ ,  $P < .05$ ], non-reinforced responses [ $F(1,9) = 7.95$ ,  $P < .05$ ], and burst responses [ $F(1,9) = 11.83$ ,  $P < .01$ ]. The decreasing trend in the numbers of reinforced responses did not reach statistical significance ( $P > .05$ ). While SKF83959-treated rats exhibited significantly lowered peak rates [ $F(1,9) = 6.00$ ,  $P < .05$ ], the peak time was unaffected by drug treatment. One of the 6 rats in the drug group in either the FI30 or DRL10 task did not respond on SKF83959 injection ([supplementary Table 1](#)).

Figure 3 illustrates the effects of SKF 83959 on the protein expression of CaMKII in the 4 specified brain regions of rats from the FI30 and DRL10 groups, respectively. For the FI30 task, a significant decrease in phospho-CaMKII (pCaMKII) protein level was found in the NAc of SKF83959-treated rats compared with that of the vehicle-treated rats [ $F(1,10) = 8.14$ ,  $P < .05$ ], while there was no significant difference in the PFC, DS, or Hippo between the SKF83959- and vehicle-treated rats. Meanwhile, a slight decrease in the total CaMKII protein level was also observed in the NAc, whereas a significant increase was found in the Hippo of the SKF 83959-treated rats compared with the vehicle group [ $F(1,10) = 13.29$ ,  $P < .01$ ], thus reflecting only a significant decrease in the pCaMKII/CaMKII ratio in the Hippo of SKF83959-treated rats compared with the vehicle-treated rats [ $F(1,10) = 9.14$ ,  $P < .05$ ].

For the DRL10 task, a significant decrease in the pCaMKII protein level was found in the DS of the SKF83959-treated rats

compared with that of the vehicle group [ $F(1,7) = 5.75$ ,  $P < .05$ ], but no significant difference was observed in the PFC, NAc, and Hippo between the SKF 83959- and vehicle-treated rats. Regarding the total CaMKII protein level, a significant decrease was found in the DS [ $F(1,8) = 10.09$ ,  $P < .05$ ] and Hippo [ $F(1,8) = 5.72$ ,  $P < .05$ ] of the SKF83959-treated rats compared with the vehicle-treated rats, reflecting no significant difference in the pCaMKII/CaMKII ratio between the SKF83959- and the vehicle-treated rats.

Regarding the CREB protein expression (Figure 4), for the FI30 task, a significant decrease in phospho-CREB (pCREB) protein level was found in the NAc [ $F(1,10) = 6.60$ ,  $P < .05$ ] and Hippo [ $F(1,10) = 19.08$ ,  $P < .01$ ] of SKF83959-treated rats compared with that of the vehicle-treated rats. In terms of total CREB protein level, there was a significant decrease in the NAc [ $F(1,10) = 7.76$ ,  $P < .05$ ] and Hippo [ $F(1,10) = 6.97$ ,  $P < .05$ ] of SKF83959-treated rats compared with that of the vehicle-treated rats. However, the pCREB/CREB ratio only significantly decreased in the Hippo of SKF83959-treated rats compared with the vehicle-treated rats [ $F(1,10) = 5.22$ ,  $P < .05$ ].

For the DRL10 task, a significantly higher pCREB protein level was only found in the NAc of the SKF83959-treated rats compared with the vehicle-treated rats [ $F(1,9) = 14.03$ ,  $P < .01$ ]. Regarding the total CREB protein level, there were no significant differences across the examined brain regions between the SKF83959 and vehicle treatments. And the pCREB/CREB ratios revealed significant increases in the PFC [ $F(1,9) = 12.51$ ,  $P < .01$ ] and NAc [ $F(1,9) = 6.75$ ,  $P < .05$ ] of the SKF83959-treated rats compared with the vehicle-treated rats.

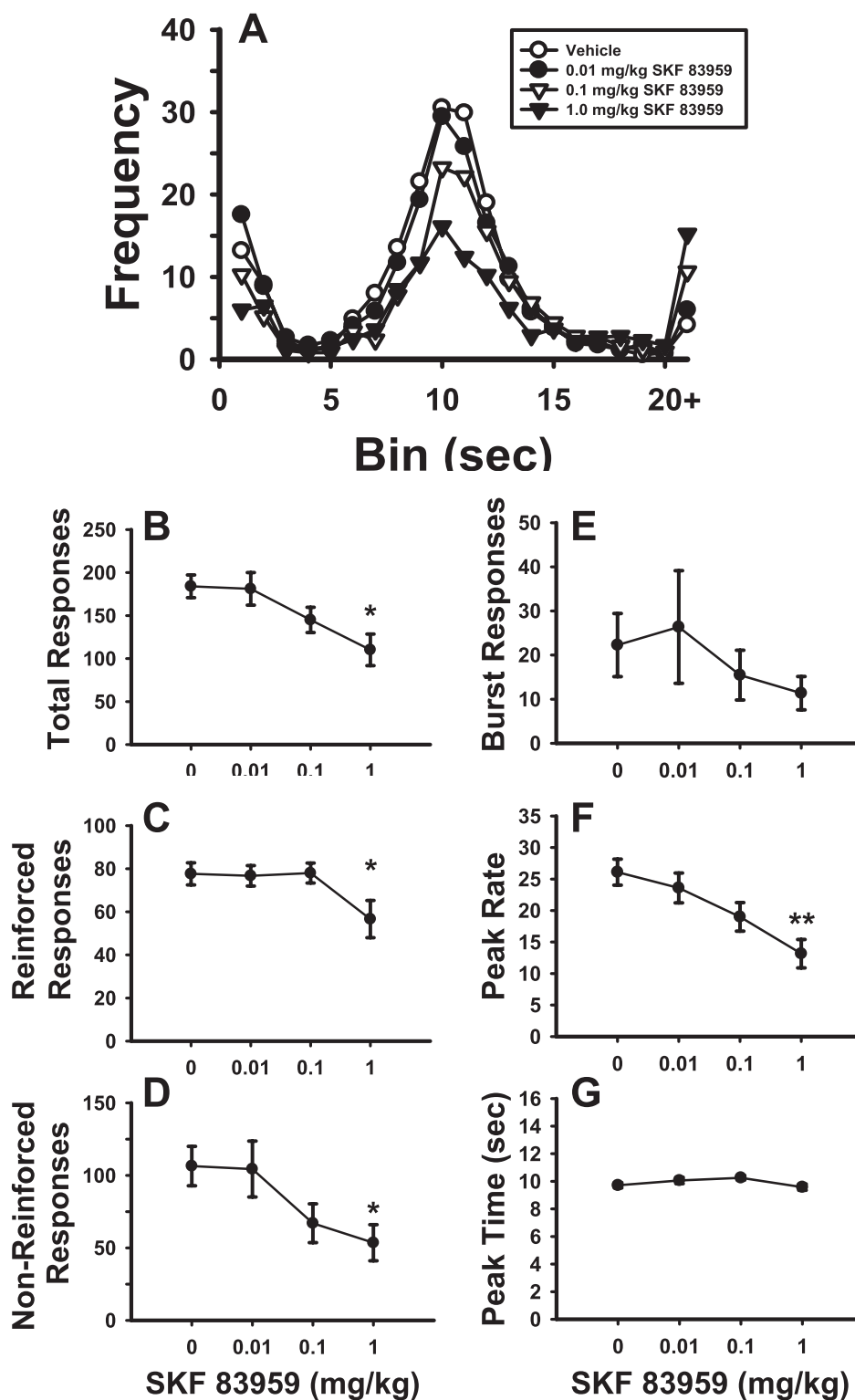
Regarding the correlational analysis, on the behavioral level, the administration of SKF83959 was found to be negatively correlated with the numbers of total responses on both of the FI30 [ $r(10) = -0.838$ ,  $P < .01$ ] and DRL10 [ $r(9) = -.674$ ,  $P < .05$ ] groups. On the protein level, correlation analyses were conducted between the levels of the pCaMKII and pCREB across brain regions under the assumption that the acute administration of SKF 83959 affected protein phosphorylation in the brief time period before tissues were collected. In the DS, the levels of pCaMKII and pCREB were positively correlated in the FI [ $r(10) = 0.702$ ,  $P < .05$ ] and DRL [ $r(9) = 0.725$ ,  $P < .05$ ] groups. In the NAc of the FI30 group, the levels of pCaMKII and pCREB were positively correlated with each other [ $r(10) = 0.690$ ,  $P < .05$ ]. In the NAc of the DRL10 group, the levels of pCaMKII and pCREB were positively correlated with each other [ $r(10) = 0.883$ ,  $P < .01$ ]. In the PFC and Hippo, no significant correlation between pCaMKII and pCREB was detected in either the FI30 or DRL10 group.

## Experiment 3

The dose effects of SKF83959 on spontaneous locomotor activity are shown in Figure 5. In the top panel, for the within-session analysis, there was only a significant main effect of blocks on locomotor activity [ $F(5,60) = 89.3$ ,  $P < .001$ ]. Despite a trend of dose-related increment visualized in blocks 2–5, neither the dose effect nor the interaction test was significant [ $F(2,12) = 1.148$ ,  $P = .350$ ] and [ $F(10,60) = 1.131$ ,  $P = .355$ ], respectively. A lack of significant dose effect of SKF83959 on locomotor activity was also confirmed by the total distance measured in the 30-minute test ( $P > .05$ ; bottom panel).

## Discussion

The present study is the first, to our knowledge, to evaluate the effects of SKF83959 on timing-relevant operant behaviors (i.e.,



**Figure 2.** Dose effects of SKF83959 on differential reinforcement of low-rate response 10-second schedule (DRL10) behavior (Experiment 1) as shown by inter-response time (IRT) distribution (A), total number of responses (B), the number of reinforced responses (C), the number of nonreinforced responses (D), the number of burst responses (E), peak rate (F), and peak time (G). Asterisks denote significant differences between the indicated dose treatment and the vehicle control by post hoc tests: \* $P < .05$ , \*\* $P < .01$ .

maintained on FI and DRL schedules) in rats. The drug is more potent in affecting FI30 behavior than DRL10 behavior in terms of the dose effect on total responses. Compared with the vehicle, the acute administration of SKF83959 at 1.0 mg/kg reduced the

total responses on the FI30 schedule by approximately sixfold and decreased that on the DRL10 schedule by approximately twofold. The results highlight task-dependent differences in operant response to the FI and DRL schedules of reinforcement.

**Table 1.** Effects of 1 mg/kg SKF83959 on FI30 and DRL10 behavior (Experiment 2: behavioral pharmacology)

	Vehicle control	SKF83959 (1 mg/kg)
<b>FI30 behavior</b>		
Total responses	618±83	101±67***
Reinforced responses	57±1	24±8**
PRP (sec)	20.7±1.2	37.4±3.7**
<b>DRL10 behavior</b>		
Total responses	187±19	77±33*
Reinforced responses	74±5	43±16
Non-reinforced responses	112±17	34±21*
Burst responses	23±5	5±3**
Peak rate	26.0±3.5	11.2±4.7*
Peak time	9.7±0.4	10.1±0.4

\* $P < .05$ , \*\* $P < .01$ , \*\*\* $P < .001$  (compared with vehicle control).

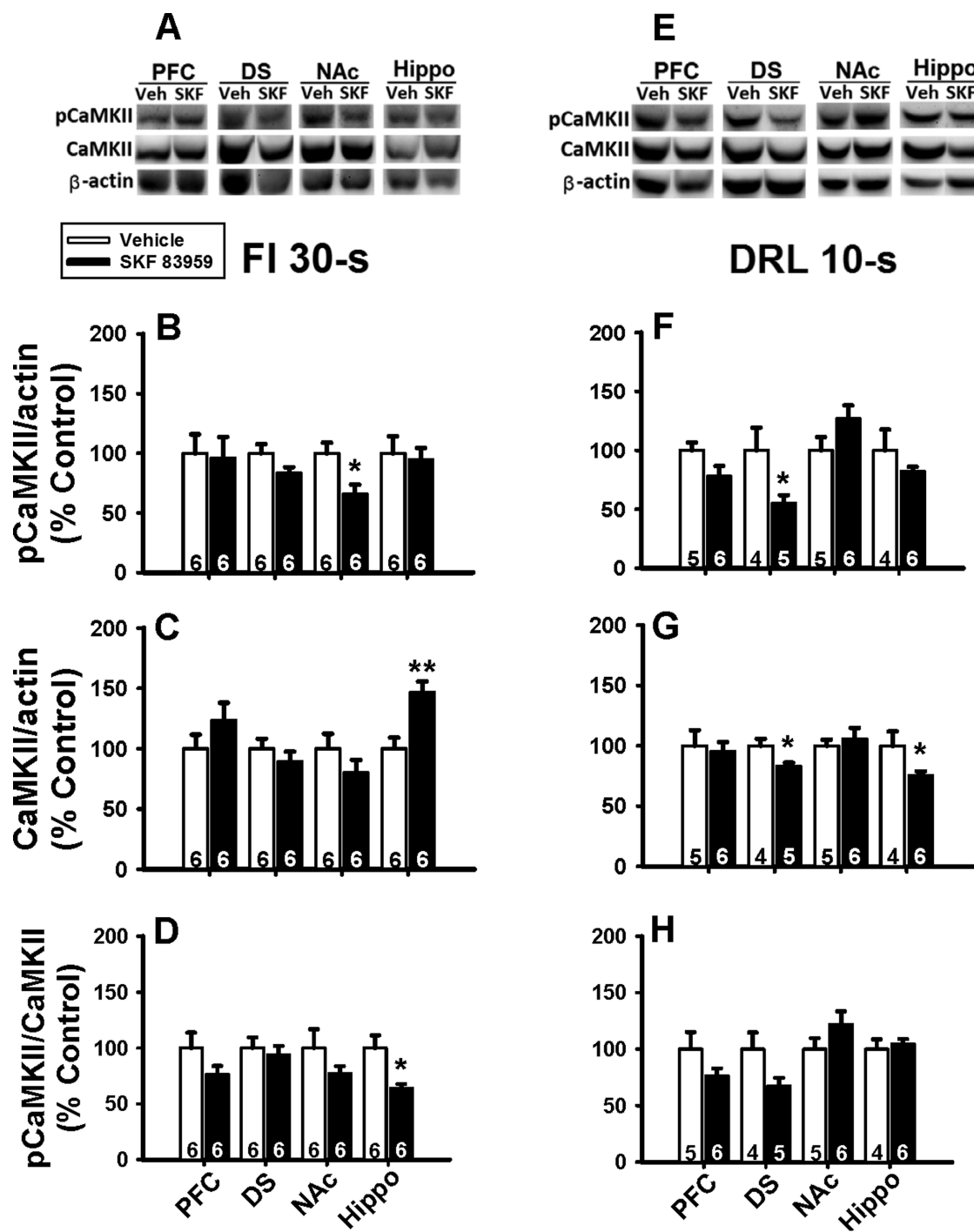
In primates, Platt et al. (2000) reported that SKF83959 injections dose-dependently reduced the operant response rates on a FI 3-minute shock termination task; the administration of 0.3 mg/kg SKF83959 reduced the response rates by nearly twofold compared with the control, whereas that of 3.0 mg/kg SKF83959 almost completely diminished the response rates. These data are consistent with our findings on the response rate reduction on the FI30 schedule in appetitive conditioning. As an index to verify the acquisition of FI behavior, the PRP duration of rats trained on the present FI30 schedule was increased from a few seconds (e.g., 2–3 seconds) at the beginning of training to a duration of approximately 20 seconds later in a stable performance, compatible with the notion that PRP increased with increasing durations of FI schedules (Chung and Neuringer, 1967; Shull, 1970). The SKF83959-prolonged PRP on the FI30 schedule was consistently observed in Experiments 1 and 2, indicating that the drug may reduce the subject's intrinsic interference with the sense of duration with regard to reinforcement contingency. The measure of PRP or the latency to the first response made during the interval is an important empirical referent of the temporal control involved in the performance of FI behavior (Daniels and Sanabria, 2017). Moreover, the involvement of DA-subtyped receptors in FI schedule-controlled behavior using typical natural reinforcer has been demonstrated in studies that included the pharmacological manipulation of systemic injection or intra-NAc infusion of drugs selectively acting on D1 and/or D2 receptors (Cory-Slechta et al., 1996; Cory-Slechta and Bare, 1997; Jaszyna et al., 1998; Areola and Jadhav, 2001). The release of DA in the NAc is associated with the operant response to FI task (Cousins et al., 1999; Richardson and Gratton, 2008). Despite that these abovementioned reports supported the contribution of the NAc DA to FI response, dopaminergic signaling involved in this behavior, as well as DRL behavior, has not been scrutinized.

The peak time measured in the DRL behavior was used to elaborate the relationship between the drug treatment and the timing process (Cheng and Liao, 2005; Cheng et al., 2006, 2008; Liao and Cheng, 2007; Chiang et al., 2015). In this study, SKF83959 did not affect the peak time of the DRL10 behavior at all tested doses, but it had profound effects on the other response-related measures and the peak rate. The lack of effect on DRL peak time displays the uniqueness of SKF83959 compared with those described for FI30 in terms of timing process, thereby indicating that the temporal processes involved in these 2 behaviors cannot be the same. Given the different reinforcement contingencies set in FI and DRL schedules, the subjects trained by FI

task normally perform increasing response rates within the intervals, whereas those trained by DRL task display a relatively low rate of response because a slower response increases the chance to obtain the reinforcer. Although the process of interval timing could be required in the performance of either schedule-controlled behavior, “cost” exists in getting the timing wrong as any response made prior to the criterial interval fully elapsed in the DRL (rather than FI) task. Any “premature” response with the IRT shorter than the criterial interval is not reinforced and leads to an interval reset in the DRL task. Thus, in addition to timing process, behavioral inhibition is required in the performance of DRL behavior; conversely, this is not the case for FI behavior. The lack of drug influence on the peak time of DRL10 behavior has been reported in the acute treatment of selective DA-subtyped receptor agonists, including SKF38393 (a D1 receptor agonist), quinpirole (a D2/D3 receptor agonist), bromocriptine (a D2/D3 receptor agonist), and PD168077 (a D4 receptor agonist) (Chiang et al., 2015). Notably, SKF83959 with a greater impact on peak rate differs from the 4 aforementioned DA receptor agonists, which had little influence on this measurement. Intriguingly, the effects of SKF83959 on the DRL10 task were similar to those reported for SCH23390 and raclopride, which are selective D1 and D2 receptor antagonists, respectively (Liao and Cheng, 2005; Cheng and Liao, 2007). Thus, the mechanism that distinguishes the differences between SKF83959 and other drugs that act on DA subtype receptors is complex and may be related to the unique pharmacological properties from SKF83959-coupled intracellular signaling pathways.

CaMKII is an abundant protein inside neurons. The activation of this protein is regulated by calcium ions. Studies on CaMKII originally focused on its functions in linking calcium signals to synaptic plasticity and long-term potentiation or long-term depression in the hippocampus during the N-methyl-D-aspartate receptor signaling cascade (Fink and Meyer, 2002). CaMKII plays a critical role in learning and memory; however, the mechanisms by which protein kinase induced the reward-related behaviors remain elusive. CaMKII in the NAc is known to modulate behavior associated with drug reward (e.g., Loweth et al., 2010), but the role of this protein kinase in operant response maintained by natural reinforcer remains unclear. Nonetheless, Wiltgen et al. (2007) investigated the effects of striatal CaMKII activation by using transgenic mice that constitutively expressed active CaMKII in the striatum through the behavioral test of classical and instrumental conditionings. Their results suggested that striatal CaMKII is critical in forming associations between Pavlovian cues and instrumental responses to obtain reinforcers (Wiltgen et al., 2007). However, the study did not report that these striatal CaMKII changes might be further attributed to the NAc and/or DS. As shown in the present study, pCaMKII protein level in the NAc under the treatment of SKF83959 at 1.0 mg/kg increased in the DRL10 group but decreased in the FI30 group. SKF83959 also decreased the pCaMKII in the DS of the DRL10 group. Our findings support the notion that striatal CaMKII is involved in the instrumental conditioning. Moreover, the present results indicate the different regulatory effects of SKF83959 on pCaMKII protein level in various striatal subareas; this protein could be involved in the processing of different types of schedule-controlled behavior.

Repeated injections of SKF83959 (0.4 mg/kg) remarkably reduced the total CaMKII protein level rather than its phosphorylation status in the NAc (Perreault et al., 2010). In comparison, the pCaMKII level in the NAc markedly increased after the acute injection of SKF83959 at 3 mg/kg combined with quinpirole (Rashid et al., 2007). The pCaMKII protein level in the striatum



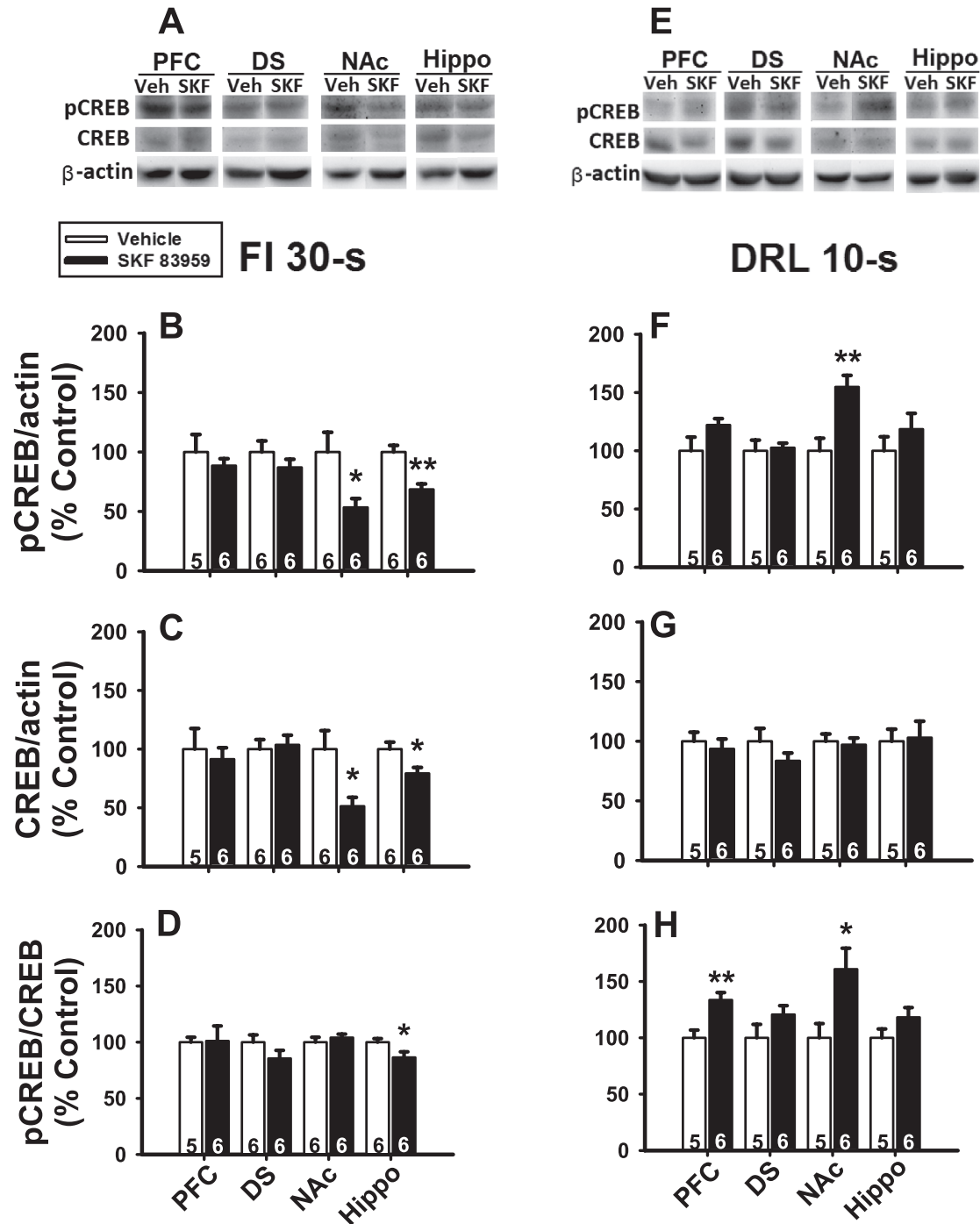
**Figure 3.** Representative immunoblots and quantitative analyses of phospho-calcium-/calmodulin-dependent protein kinase II (pCaMKII) and calcium-/calmodulin-dependent protein kinase II (CaMKII) expression levels in the medial prefrontal cortex (PFC), dorsal striatum (DS), nucleus accumbens (NAc), and hippocampus (Hippo) in the vehicle- and SKF83959-treated rats after behavioral tests on fixed-interval 30-second schedule (FI30) (A–D) and differential reinforcement of low-rate response 10-second schedule (DRL10) (E–H). The number in each bar represents the sample size of the corresponding group being analyzed. Asterisks denote significant differences between drug treatment and vehicle control: \* $P < .05$ ; \*\* $P < .01$  (Experiment 2).

increased significantly at 30, 60, and 90 minutes after injection with SKF83959 at 1.0 mg/kg (Ng et al., 2010). These reported findings are consistent with our finding that pCaMKII in the NAc of rats in the DRL10 group was activated 30 minutes after treatment with SKF83959 at 1.0 mg/kg. However, the pCaMKII level in the NAc of the FI30 group decreased with SKF 83959 treatment. The cause of these opposite effects of SKF83959 could be the other signaling cascade, such as calcineurin (Savica and

Benarroch, 2014), which may be involved in the drug-altered FI and DRL behaviors (as shown below).

CREB regulates the neuroplasticity underlying learning and memory, and its activity can be affected by a complex array of neural signals from pathways involved in CaMKs, cAMP-PKA, and MAPK/ERK (Carlezon et al., 2005; Nishi et al., 2011). CaMKII phosphorylates CREB at serine-133 and Serine-142 residues (Carlezon et al., 2005). The phosphorylation of serine-133 induces





**Figure 4.** Representative immunoblots and quantitative analyses of phospho-cyclic AMP response element binding protein (pCREB) and cyclic AMP response element binding protein (CREB) expression levels in the medial prefrontal cortex (PFC), dorsal striatum (DS), nucleus accumbens (NAc), and hippocampus (Hippo) in the vehicle- and SKF83959-treated rats after behavioral test on fixed-interval 30-second schedule (FI30) (A–D) and differential reinforcement of low-rate response 10-second schedule (DRL10) (E–H). The number in each bar represents the sample size of the corresponding group being analyzed. Asterisks denote significant differences between drug treatment and vehicle control: \* $P < .05$ ; \*\* $P < .01$  (Experiment 2).

CREB dimerization and activates the subsequent gene expression (Sun et al., 1994). The present results showed that the decrease in the serine-133 phosphorylation of CREB in the NAc of the FI30 group was positively correlated with pCaMKII in the NAc. The positive correlation between pCREB and pCaMKII in the NAc also appeared in the DRL10 group. This result supported the notion that CaMKII–CREB-mediated signaling is involved in the cellular mechanism of SKF83959 on operant behaviors. Moreover, the protein levels altered by SKF83959 were found mainly in the DS and NAc, whereas relatively few protein changes were found

in the PFC and Hippo. These results suggested that the striatum is likely the primary targets of SKF83959 treatment to affect FI and DRL behavior with different profiles of CaMKII–CREB-mediated signaling. However, further studies with appropriately controlled experiments are needed to confirm this inference, because the synergistic regulation on CREB by other kinases in the dysregulation of motivated behavior has been noted, for example, in drug addiction (Muschamp and Carlezon, 2013).

SKF83959 activates CaMKII through the D1–D2 receptor heteromer-mediated calcium signaling but not through the

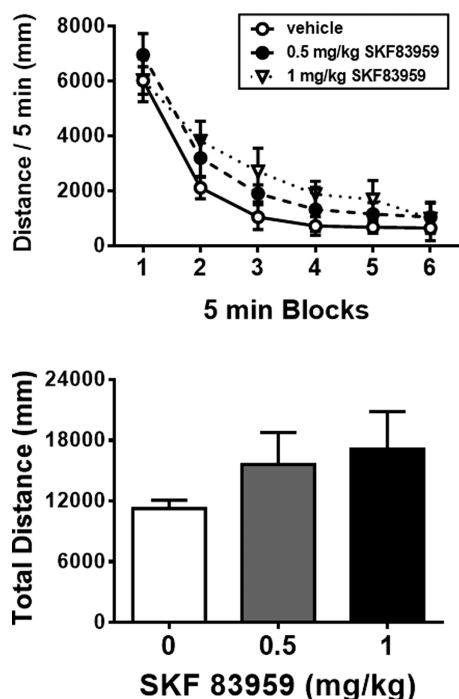


Figure 5. Dose effects of SKF83959 on spontaneous locomotor activity: (top) distance measured in 5-minute blocks and (bottom) total distance during the 30-minute test (Experiment 3).

N-methyl-D-aspartate receptors (Lee et al., 2004; Rashid et al., 2007; Ng et al., 2010; Perreault et al., 2010). Besides CaMKII, protein phosphatase 2B (calcineurin) is a downstream molecule involved in calcium signaling under SKF83959 treatment (Liu et al., 2009; Ma et al., 2009b). A high-density of D1-D2 receptor heteromer was expressed in the rat striatum compared with other DA-associated brain regions (Lee et al., 2004; Ng et al., 2010; Hasbi et al., 2020b). Thus, given that the coordination of protein phosphorylation and dephosphorylation plays an important cellular mechanism role responsive to different neuropsychiatric behaviors (Woolfrey and Dell'Acqua, 2015), the current results of SKF83959-altered operant responding are possibly associated with different drug-induced profiles of protein phosphorylation, as observed in the NAc and DS. Further investigations are needed to illustrate the mechanism of D1-D2 heteromer receptor-coupled signaling in SKF83959-altered operant behavior. One possible approach is to pharmacologically inhibit or reverse the effects of SKF83959 by a selective agent that can exert functional antagonism on D1-D2 receptor heteromer such as the TAT-D1 peptide (Shen et al., 2015a, 2015b; Hasbi et al., 2018). For example, D1-D2 heteromer activated by SKF83959 can inhibit the canonical DA homomer signaling pathway, thereby leading to the attenuation of cocaine- and food-seeking behaviors, which can be reversed by TAT-D1 peptide (Hasbi et al., 2018). Understanding how the D1-D2 receptor heteromer engaged in the distinctive features of operant behavior maintained on different schedules of reinforcement may help elucidate the cellular or molecular substrate of the motivational/cognitive control involved in a specific reinforcement contingency of operant behavior. With the advances in the neurobiology of the known DA receptor heteromer, the D1-D2 receptor heteromer may become a potential target for neuropsychiatric disorders associated with motivational/cognitive control impairment (Perreault et al., 2014; Andrianarivelo et al., 2019).

The locomotor activity is sensitively affected by drugs that alter brain DA transmission. The compounds that enhance

brain DA transmission include the following: amphetamine, cocaine, and apomorphine (Smith, 1965; Cole, 1978; Castro et al., 1985); D1 receptor full agonists (i.e., SKF82958 and SKF81297); and D1 receptor partial agonists (i.e., SKF75670, SKF77434, and SKF38393) (Meyer and Shults, 1993; Halberda et al., 1997; Schindler and Carmona, 2002; Desai et al., 2005). However, mixed results were obtained by studies on the effects of D2 receptor agonists on locomotor activity, including a decrement by quinpirole (Mattingly et al., 1993; Halberda et al., 1997) and biphasic effects by bromocriptine (Eilam and Szechtman, 1989; Hoffman and Wise, 1992). Moreover, the locomotor activity was consistently reduced by both D1 and D2 receptor antagonists (Hoffman and Beninger, 1985; Hillegaart and Ahlenius, 1987; Schindler and Carmona, 2002; Collins et al., 2010). A pharmacological study by Deveney and Waddington (1995) showed that rats injected with SKF83959 (dosing from 0.01 to 1.25 mg/kg) produced no effect on locomotor activity despite the increases in rearing at the higher tested doses. Peacock and Gerlach (2001) reported that SKF83959 did not induce any motor unrest in primates. Although recent studies reported SKF83959-increased locomotor activity in rats, such effect was manifested under certain paradigms of drug administration in rats (Perreault et al., 2010; Shen et al., 2015b). Interestingly, amphetamine-induced locomotor sensitization was pharmacologically reversed by a non-effective dose of SKF83959 (Shen et al., 2015b). A similar pharmacological antagonism by SKF83959 was observed in experiments with cocaine-induced locomotor sensitization (Hasbi et al., 2018). Considering the null result of this study, discrepancies between studies may be attributed to different procedures used in testing the effects of SKF83959 on locomotor activity. Nonetheless, in the present study, the locomotor activity was preserved at doses that reduced operant response, thereby indicating that the motor functions engaged during ambulation are anatomically distinct from those involved in lever pressing (i.e., head and forelimb skilled motor control). Behaviorally, locomotor activity and operant response are categorized in different paradigms, such as unconditioned and associative learning, respectively. The effects of a DA-related drug on behavior could depend on the motor kinetics required by the tasks (Ettenberg et al., 1981).

An issue to consider is the fact that water-restricted rats were used as the subjects in the present study. A possible concern is whether the current results could be generalized to those reported by other relevant studies, in which food-restricted subjects were used, considering that food restriction has well-documented effects on DA systems and the behavioral effects of drugs that act on DA systems (Cardinal and Everitt, 2004; Nunes et al., 2013; Carr, 2020). Besides increasing motivation to respond for water, fluid deprivation possibly impacts the consumption of food, because the majority of food intake occurs during or shortly after water is made available. A water-deprived rat is hungry because of self-imposed food deprivation (Herberg and Stephens, 1977; Toth and Gardiner, 2000). Thus, even if bodyweights are maintained under this procedure, the behavioral effects of SKF83959 are most likely being evaluated on rats that are in a self-imposed state of food restriction. While operant behavior is generally run on rats with a certain level of restriction-related motivation, this study used the FI and DRL task to mainly evaluate the drug effects on distinct components of timing-associated reinforcement contingency involved in these 2 tasks. As for the motivation to consume the reinforcer being concerned, further investigation using a specific operant task (e.g., with a progressive ratio schedule) is required to evaluate whether

SKF83959 specifically affects the effort-related motivation to pursue a reinforcer.

In conclusion, the present study demonstrated that task-dependent differences in SKF83959-altered operant response were manifested by rats in FI30 and DRL10 schedules of reinforcement. Changes in protein phosphorylation of CaMKII and CREB by SKF83959 depended on behavioral task and brain region. That the locomotor activity was preserved at doses that disrupted operant response indicates distinct neurobiological bases engaged in the behavioral functions of these 2 paradigms. These findings provide important insight into the neuropsychopharmacology of SKF83959 and further suggest the involvement of striatal CaMKII-CREB signaling pathway in the operant behavior altered by this agent.

## Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Interest Statement: None (all authors).

## Author Contributions

Conceptualization and experimental design: CCC and RML; behavioral experiment: PPL and RML; western-blot experiment: PPL and CCC; data curation and statistical analysis: PPL, CCC, and RML; writing, original draft: PPL and RML; writing, editing, and paper revision: CCC and RML; funding acquisition: CCC and RML.

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