國立政治大學心理學研究所

碩士論文

SKF 83959 對時距有關的操作式制約行為之影響效果及其神經機制

The Effects of SKF 83959 on Time-Based Operant Behaviors and the Underlying Neural Mechanisms

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摘要

近年來與多巴胺功能相關的研究，除探討各型多巴胺受體引發細胞內訊息傳遞機制，亦開始檢測其次級受體之間如何互動及其功能作用為何。近期研究在大腦中的紋狀體發現了由第一型多巴胺受體（D_1R）與第二型多巴胺受體（D_2R）所共同組成的雜二聚體（heterodimer），其活化會激發一連串有別於傳統多巴胺傳導的訊息傳遞路徑，其中包括經磷脂酶 C 調控的磷酸肌醇之水解，以及後續的細胞內鈣離子釋放。目前，此 D_1-D_2 雜二聚體在活體（*in vivo*）層級的功能不明，仍尚待釐清。因此，本研究以一個屬於 D_1-D_2 雜二聚體致效劑的 SKF 83959 藥物，檢測其對大鼠於兩項與時距有關的操作式制約行為表現（FI 30-s 與 DRL 10-s）和自發性活動量之影響，並測量四個大腦多巴胺相關區域的特定蛋白質表現受此藥物行為作用之影響。本研究結果發現，隨著 SKF 83959 藥物處理的劑量增加，大鼠於 FI 30-s 以及 DRL 10-s 作業上的反應率受到了顯著的降低，然而自發性活動量並未受到藥物效果影響。此項結果顯示 SKF 83959 可能對獲取酬賞物相關的內在動機歷程有影響，而不是因藥物引起運動失能之效。在生化測試蛋白質表現量的結果，SKF 83959 對背側紋狀體以及伏隔核中的 pCaMKII、PKA、及 pCREB 引發了較明顯的蛋白質表現量變化，在前額葉與海馬迴則未有此效。本研究另外嘗試藉由藥理拮抗的實驗，檢測多巴胺受器拮抗劑是否能反轉 SKF 83959 藥物對行為表現。實驗結果大致顯示單獨施打 SCH 23390 以及共同施打 SCH 23390 與 eticlopride 的前處理，無法反轉 SKF83959 對於操作式制約行為表現的影響；然而，低劑量的 eticlopride 對回復 SKF 83959 所引發的低反應率有部份藥理反轉的效果。綜合以上結果，SKF 83959 會有不等程度的影響本研究所採之兩種時距有關的操作式制約行為，其可能涉及大腦紋狀體內 CaMKII/CREB 的生化作用。未來研究可考慮直接操弄細胞內蛋白質的表現量，或者觀測 D_1-D_2 雜二聚體於活體內的活動，以提供更多關於此 D_1-D_2 雜二聚體參與個體的行為功能與其相關神經機制。

關鍵字：多巴胺受體、D_1-D_2 雜二聚體、SKF 83959、操作式制約行為、FI 30 秒作業、DRL 10 秒作業

Abstract

As the functions and signaling mechanisms of dopamine (DA) receptor subtypes remain popular topics of research, recent studies have also begun to investigate the interactions between different subtypes of receptors. The formation of DA D_1-D_2 receptor complexes was discovered in the striatum, whose activation leads to a novel signaling pathway via phospholipase C-mediated phosphoinositide hydrolysis, followed by intracellular calcium release. As the *in vivo* functional role of this D_1-D_2 receptor heteromer remains to be elucidated, the present study investigated the effects of SKF 83959, a proposed D_1-D_2 heteromer-selective agonist, on the performance of schedule-controlled behaviors (FI 30-s and DRL 10-s), locomotor activity, and the expression of related proteins in four terminals of the mesocorticolimbic DA system, which included the prefrontal cortex (PFC), dorsal striatum (DS), nucleus accumbens (NAc), and dorsal hippocampus. The administration of SKF 83959 was found to reduce the response rates of FI 30-s and DRL 10-s in a dose-dependent manner, whereas the locomotor activity was not affected. This suggests that SKF 83959 may have affected the processes of intrinsic motivation to obtain the reinforcers, rather than motor control. In respective to protein expression, SKF 83959 induced prominent changes in the levels of pCaMKII, PKA, and pCREB in the DS and NAc relative to the PFC and hippocampus. Experiments of pharmacological antagonism were conducted in attempts to reverse the behavioral effects of SKF 83959. The results showed that the pretreatments of SCH 23390 alone and SCH 23390 combined with eticlopride did not reverse the effects of SKF 83959 on operant behaviors. However, low dose eticlopride appeared to have a partial effect in restoring the decline in operant response rates by SKF 83959. Together, the current data showed that SKF 83959 altered the time-based operant behaviors tested to different degrees, possibly via its influence on CaMKII-CREB signaling in the NAc. Future studies that

manipulate the activation of intracellular proteins or quantify the levels of D_1-D_2 heteromer activation may provide more information regarding the *in vivo* activation mechanisms of D_1-D_2 heteromers.

Keywords: dopamine receptor, D_1-D_2 heteromer, SKF 83959, operant behaviors, FI 30 sec schedule, DRL 10 sec schedule.

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Introduction

The Behavioral Functions of DA: A Historical Overview

The theory of operant conditioning by Skinner (1938), which described the reinforcement of actions with positive outcomes, has led to the search for reward-related regions in the brain, to better understand the mechanisms of operant learning. Olds and Milner (1954) implanted electrodes into rat brains and tested their operant responses for electrical stimulation, in a paradigm later known as the intracranial self-stimulation (ICSS). It was found that electrodes implanted in the septal area, located in the central forebrain beneath the corpus callosum, corresponded with increased responding for electrical stimulation during the acquisition phase (Olds & Milner, 1954). Olds and Olds (1963) aimed to more precisely identify the brain loci involved in positive and negative reinforcement by implanting electrodes into different brain regions across animal subjects and testing their responses on self-stimulation tasks; it was found that the implantation of electrodes in the medial forebrain bundle (MFB) was highly correlated with increased responding for electrical stimulation, in other words, with positive reinforcement. As ascending norepinephrine (NE) axons were observed within the MFB, and the pharmacological manipulations of NE transmission with monoamine oxidase inhibitors (eg. d-amphetamine) and inhibitors (eg. reserpine) increased or decreased animal response rates on ICSS respectively, the catecholamine hypothesis, which suggested that NE neurons in the MFB are related to the reinforcing effects of the ICSS, was proposed to explain the correlation between NE transmission and ICSS performance (Fibiger, 1978). During the popularity of the catecholamine hypothesis, DA was considered as a precursor molecule in NE synthesis (Wise, 2008). In their studies, Carlsson, Lindqvist, and Magnusson (1957) administered 3,4-dihydroxyphenylalanine (DOPA; a precursor in DA and NE syntheses) to mice, and found the

drug to counteract the motor deficits induced by reserpine. Carlsson, Lindqvist, Magnusson, and Waldeck (1958) further reported that such observations were related to the levels of DA instead of NE in the brain. Together, these findings led to the proposal that DA is not a mere reaction intermediate during NE synthesis; it may function as a neurotransmitter on its own (Carlsson et al., 1958).

The monoamine oxidase inhibitors and catecholamine inhibitors mentioned earlier do not only affect NE transmission, but the DA system as well. Hence subsequent studies investigated the effects of NE- or DA-selective antagonism on operant responding. The antagonism of brain noradrenergic transmission was found to debilitate the general abilities to perform on self-administration tasks in animals rather than affecting their reward-mediated responses (Wise, 2008). On the contrary, the blockade of DA transmission with pimozide decreased the operant responding for food without affecting the general abilities to perform on the task (Wise, Spindler, De Wit, & Gerberg, 1978). As pimozide appeared to block the rewarding effects of food, the anhedonia hypothesis was proposed to suggest an important role of brain DA in the processing of pleasure and reward-mediated motivation (Wise et al., 1978; Wise, 1982). Since then, research on reward-mediated brain regions has begun to focus on DA transmission in the brain. In recent years, studies on the DA system have identified its involvement in functions beyond the processing of hedonic stimuli; the DA system is now believed to possess functional roles in a variety of activities, spanning from motivated behaviors, motor movement, effort-related decision making, to habit formation, reward prediction, and interval timing (Salamone & Correa, 2012; Schultz, Dayan, & Montague, 1997; Meck, Penney, & Pouthas, 2008). As selective agonists and antagonists for DA receptors (DARs) become available, it is possible to experimentally activate specific dopaminergic pathways and discover their impacts on animal

behaviors. The emergence of studies on the various aspects of DA functions offers insights to the underlying mechanisms of motor movement, operant conditioning, cognitive processing, as well as the future understanding of pathological problems like drug addiction, Parkinson's disease (PD), and schizophrenia. Particularly, the present proposed study will focus on the role of DA in reward-mediated operant behaviors.

DA Receptors and Signaling Pathways

The transmission of DA is mediated via DARs on the cellular level. Research on the DA system has identified 5 subtypes of G-protein-coupled DARs. The D_1 and D_5 receptors were categorized into the D_1-like family based on their biochemical properties to stimulate the downstream enzyme adenylyl cyclase (AC) via stimulatory α subunits of G proteins ($G_{\alpha s/olf}$); the D_2, D_3, and D_4 receptors were categorized as D_2-like based on their properties to inhibit AC via inhibitory α subunits of G proteins ($G_{\alpha i/0}$) (Kebabian & Calne, 1979; Missale, Nash, Robinson, Jaber, & Caron, 1998). The two classes of DARs are pharmacologically and biochemically distinct, and they are differentially distributed in the brain (Missale et al., 1998).

The differential modulation over AC by D_1- and D_2-like receptor activation leads to different downstream signaling cascades. The activation of AC via D_1-like receptors in the striatum leads to the formation of the second messenger cyclic adenosine 3'5'monophosphate (cAMP), which continues on to activate protein kinase A (PKA), a cAMP-dependent protein kinase (Stoof & Kebabian, 1981). PKA will phosphorylate the DA- and cAMP-regulated phosphoprotein, 32 kDa (DARPP-32), which will go on to inhibit protein phosphatase-1 and indirectly increase the activation of extracellular signal regulated kinase (ERK), which is a

protein in the glutamatergic N-methyl-D-aspartate receptor (NMDAR) signaling cascade (Greengard, Allen, & Nairn, 1999; Valjent et al., 2005). The signaling of PKA and ERK will lead to the phosphorylation of the cAMP response element binding protein (CREB), which is a transcription factor in the nucleus that serves as an integration site for signals from DAR and NMDAR activation and regulates gene expression for synaptic plasticity (Gonzalez & Montminy, 1989; Zanassi et al., 2001; Neve, Seamans, & Trantham-Davidson, 2004; Nishi, Kuroiwa, & Shuto, 2011).

In contrast to D_1-like receptor activation, the inhibition of AC by D_2-like receptor activation is expected to result in the signaling and modulation of downstream proteins in the opposite direction (Neve et al., 2004). Nishi, Snyder, and Greengard (1997) have showed that quinpirole treatment (a D_2 receptor (D_2R)-selective agonist) reduced the phosphorylation of DARPP-32 in mice striatal slices via a calcium-dependent mechanism. Furthermore, Yan, Feng, Fienberg, and Greengard (1999) have demonstrated that quinpirole treatment induced the phosphorylation of mitogen-activated protein kinases (MAPK)/ERK and CREB in brain slices via a protein kinase C (PKC)-dependent pathway involving intracellular calcium, calcium and calmodulin-dependent protein kinase (CaMK), and DARPP-32. These findings on the signal transduction of D_1- and D_2-like receptors help to determine the relationship between the receptor functions and their respective signaling pathways. As both classes of the DARs were found to exert modulation over MAPK/ERK and CREB phosphorylation, this signaling mechanism may be related to the interaction between D_1 receptors (D_1Rs) and D_2Rs (Yan et al., 1999).

Functionally, the cAMP/PKA signaling pathway in the striatum has been suggested to be involved in the neural plasticity underlying reward-related learning; it may also be related to long-term potentiation in the hippocampus (Beninger & Miller, 1998). On the other hand, ERK

signaling is implicated in the synaptic plasticity related to learning and memory; the levels of striatal ERK activation are under the coordinated influence of the NMDARs and D_1Rs (Zanassi et al., 2001; Shiflett & Balleine, 2011).

In addition to the traditionally-identified AC pathway, Undie and Friedman (1990) discovered that the respective treatment of DA and the non-selective DA agonist apomorphine to rat brain slices could induce the accumulation of inositol phosphates in the amygdala, hippocampus, striatum, and frontal cortex; the drug-induced increases in inositol phosphate levels was blocked by the D_1R-selective antagonist SCH 23390 and not by the antagonists selective for the other receptors (such as sulpiride and atropine), suggesting that the underlying mechanism may be specifically related to D_1Rs. In examination of how much of this DA-induced phosphoinositide (PI) hydrolysis was related to the AC-coupled D_1Rs, Undie, Weinstock, Sarau, and Friedman (1994) tested the abilities of several D_1R agonists of benzazepine derivative in stimulating PI hydrolysis and AC activation using rat striatal slices. The abilities of the tested compounds in activating PI hydrolysis and AC were not correlated, suggesting that the observed PI hydrolysis may be mediated by a D_1-like receptor that is pharmacologically unique from the AC-coupled D_1-like receptors (Undie et al., 2004). These findings have inspired subsequent studies to explore possible differences in the receptor composition, intracellular signaling, and behavioral functions between the AC- and PI-linked D_1-like receptors.

DA Transmission Pathways

DA transmission in the brain is mainly classified into the nigrostriatal pathway, the mesolimbic pathway, and the mesocortical pathway according to the routes of neural projection.

First, the nigrostriatal pathway transmits DA from the substantia nigra (SNr) to the striatum, which has been shown by studies on PD to be implicated in functions of motor control and time perception. Post-mortem studies on patients with PD have reported a common degeneration of nigrostriatal DA in their brains (Kish, Shannak, & Hornykiewicz, 1988); animal lesion studies that introduced the neurotoxin 6-hydroxydopamine (6-OHDA) into the SNr or MFB have also demonstrated impaired motor movement in the animals (Deumens, Blokland, & Prickaerts, 2002). In relation to timing abilities, PD patients tended to underestimate the duration of time intervals on time estimation tasks and overestimate them on time reproduction tasks; this impairment in time perception was reduced with the treatment of the DA precursor levodopa-carbidopa (Pastor, Artieda, Jahanshahi, & Obeso, 1992).

Second, the mesolimbic pathway transmits DA from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) located in the ventral striatum and the limbic nuclei; whereas the mesocortical pathway projects DA from the VTA to the prefrontal cortex (PFC). These two pathways may be altogether referred to as the mesocorticolimbic DA pathway in discussion of the DA projection from the VTA to the limbic nuclei and PFC. The mesocorticolimbic system is comprised of interconnected neurotransmission; in addition to receiving dopaminergic input from the VTA, the NAc also receives glutamatergic input from the medial PFC (mPFC) and limbic nuclei (Pierce & Kumaresan, 2006). In response to these neural inputs, medium spiny neurons (MSNs) of the NAc project gamma amino-butyric acid (GABA) output to the ventral pallidum, VTA, and SNr, which in turn send GABAergic signals to the medial dorsal thalamus, from where glutamatergic signals to the mPFC closes this circuit (Pierce & Kumaresan, 2006).

A closer examination of the mesocorticolimbic DA pathway indicated that the striatal output can be distinguished as the direct striatonigral and the indirect striatopallidal pathways

(Parent, Bouchard, & Smith, 1984). While both pathways transmit GABAergic signals, the striatonigral pathway projects from the striatal MSNs to the medial globus pallidus, then on to the SNr, and the striatopallidal pathway projects from the striatum to synaptic relays in the lateral globus pallidus, on to the subthalamic nucleus, finally to the SNr (Albin, Young, & Penney, 1989). The striatonigral pathway is characterized by neurons selectively expressing substance P and dynorphin, whereas the striatopallidal pathway is characterized by neurons expressing enkephalin (Bertran-Gonzalez, Herve, Girault, & Valjent, 2010). Early *in situ* hybridization and retrograde labeling experiments demonstrated that the striatonigral neurons constituted nearly half of the striatal neurons, the majority of which were co-labeled with the probes for D_1R, dynorphin, and substance P, rarely with the probes for D_2R or enkephalin (Gerfen et al., 1990). The remaining neurons were co-labeled with D_2R and enkephalin probes, hence the distribution of D_1R and D_2R in the striatum was proposed to be segregated despite the presence of a fraction of striatal neurons that may express both D_1R and D_2R (Gerfen et al., 1990). The differential expression of substance P/dynorphin and enkephalin in striatal neurons has thereafter been utilized as protein markers to identify between striatonigral and striatopallidal neurons (Bertran-Gonzalez et al., 2010).

The Discovery from D_1R and D_2R Co-localization to D_1-D_2 Receptor Heteromers

While immunocytochemistry experiments using electron microscopy have reported that most of the D_1R- and D_2R-expressing MSNs in the dorsal striatum (DS) appeared segregated in distinct populations, co-localized D_1R and D_2R immunoreactivity was also observed in small numbers (Hersch et al., 1995). From *in situ* hybridization experiments, Lester, Fink, Aronin, and

DiFiglia (1993) reported a co-localization of D_1R and D_2R mRNAs in about 26% of the striatal cells. Using fluoroprobes labeling, Wong, Shetreat, Clarke, and Rayport (1999) also observed a co-localization of D_1- and D_2-like receptors in over 20% of the cultured cells from the rat striatum and NAc.

In light of the supportive evidence for D_1-D_2 receptor co-localization in the striatum, the possibility of a synergistic link between D_1Rs and D_2Rs has been proposed. Keefe and Gerfen (1995) found the co-administration of D_1R- and D_2R-selective agonists (SKF 38393 and quinpirole) to enhance the levels of immediate early gene expression in DA-depleted rat striatum compared to the administration of either receptor agonist alone. Subsequently, Lee et al. (2004) observed a novel phospholipase C (PLC)-mediated calcium increase in cultured cells co-expressing D_1R and D_2R upon the concurrent administration of D_1R- and D_2R-selective agonists (SKF 81297 and quinpirole). The rise in intracellular calcium release was not observed when the agonists were co-administered to cells expressing only D_1Rs or D_2Rs, neither when either agonist was administered alone to D_1R-D_2R co-expressing cells (Lee et al., 2004). Moreover, the dual agonist-induced calcium release could be blocked by either the D_1R-selective antagonist SCH 23390 or the D_2R-selective antagonist eticlopride (Lee et al., 2004).

The findings on D_1-D_2 receptor co-localization and synergy led to the hypothesis that the D_1R and D_2R may interact to form a heteromeric signaling unit, whose activation possibly leads to the activation of PLC-mediated calcium release rather than the classic D_1R-coupled AC pathway (Lee et al., 2004). This hypothesis of D_1-D_2 receptor heteromerization has received support from studies that used the confocal fluorescence resonance energy transfer (FRET) technique, which demonstrated co-localized D_1R and D_2R being in close proximity (within 5-7 nm of relative distance) in rat NAc in 10-20% of the NAc MSNs (Hasbi et al., 2009; Perreault et

al., 2010; Hasbi et al., 2011; Chun, Free, Doyle, Huang, Rankin & Sibley, 2013). The existence of D_1-D_2 receptor heteromers was more directly demonstrated by co-immunoprecipitation experiments on D_1-D_2 receptor co-expressing cultured cells and cells from rat striatal tissues, which showed D_1R and D_2R to be parts of a common protein complex (Lee et al., 2004). Recently, O'Dowd, Ji, Nguyen, and George (2012) have identified the interaction of glutamic acid residues in the carboxyl tail of D_1Rs with arginine residues in the intracellular loop 3 of D_2Rs as a critical step in the formation of D_1-D_2 receptor heteromers, adding more details to the understanding of D_1-D_2 receptor heteromerization.

Currently, D_1-D_2 heteromers are perceived as potential therapeutic targets for drug addiction and schizophrenia, as they were found to exhibit enhanced activity and sensitivity after repeated amphetamine treatment in rat striatum as well as in patients with schizophrenia (Perreault, Hasbi, O'Dowd, & George, 2013; Perreault et al., 2010). Thus the availability of compounds that act directly on the D_1-D_2 heteromers would be useful for possible experimental manipulations in future studies.

SKF 83959 as a Proposed Agonist for D_1-D_2 Receptor Heteromers

SKF 83959 (*R,S*-3-methyl-6-chloro-7,8-dihydroxy-1-[3'-methylphenyl]-2,3,4,5-tetrahydro-1H-benzazepine) is a compound of benzazepine derivative previously recognized as a low-efficacy D_1R partial agonist (Neumeyer, Kula, Bergman, & Baldessarini, 2003). It was found to induce animal behaviors characteristic of D_1R activation, such as grooming, vertical jaw movements, incisor chattering, and contralateral rotations in rats treated with unilateral 6-OHDA lesions (Downes & Waddington, 1993; Deveney & Waddington, 1995; Panchalingam & Undie,

2001; Makihara et al., 2007; Gnanalingham, Hunter, Jenner, & Marsden, 1995). However, it exhibited antagonist-like effects biochemically on DARs in failing to stimulate AC activity and actually inhibiting DA-induced AC activity (Arnt, Hyttel, & Sanchez, 1992). These observations led to the speculation that the atypical mechanism of SKF 83959 may be related to a DA signaling pathway that is independent of AC. In support of this idea, Panchalingam and Undie (2001) reported an induction of PLC-mediated PI hydrolysis in rat and monkey brain slices upon SKF 83959 treatments; hence SKF 83959 was suggested to act on the previously reported PI-linked D_1-like receptor in the brain (Undie & Friedman, 1990; Undie et al., 2004). Subsequently, Rashid et al. (2007) detected a modest calcium signal when SKF 83959 was applied to D_1-D_2 receptor co-expressing human embryonic kidney (HEK) cells in vitro; the calcium signal was actually enhanced when SKF 83959 was co-administered with the D_2R agonist quinpirole. Radioactively-labeled guanosine triphosphate (GTP γ S) assays showed the administration of SKF 83959 to significantly increase the incorporation of labeled GTPs into $G_{q/11}$ proteins, suggesting that the PI-linked D_1-like receptor may transmit neural signals via G_q proteins in contrast to G_s or G_i (Rashid et al., 2007). It is worth noting that the activation of $G_{q/11}$ by SKF 83959 was prevented by the administration of either SCH 23390 or raclopride, which further demonstrated the critical roles of concurrent D_1R and D_2R stimulation in the activation of the $G_{q/11}$-linked signaling cascade (Rashid et al., 2007). In summary of the above findings, the PI-linked D_1-like signaling unit was hypothesized to be composed of the heterodimerized D_1-D_2 receptors, which can be activated by the novel compound SKF 83959, leading to the stimulation of $G_{q/11}$ proteins, PLC-mediated PI hydrolysis, and finally the release of intracellular calcium followed by the activation of the calcium/calmodulin-dependent protein kinase II (CaMKII) (George & O'Dowd, 2007; Hasbi, O'Dowd, & George, 2010).

However, a contradictory finding has been reported by Chun et al. (2013), in which they found SKF 83959 to antagonize calcium mobilization in D_1-D_2 receptor co-expressing HEK cells and possess high binding affinities to several G-protein coupled receptors other than the D_1R or D_2R. Chun et al. raised doubts regarding the biochemical mechanism and receptor specificity of SKF 83959 as a D_1-D_2 receptor heteromer-specific agonist, and suggested that the D_1-D_2 receptor-mediated calcium signaling may not be entirely heteromer-specific and may involve other downstream signaling pathways.

While the biochemical mechanism of SKF 83959 still requires further investigation and careful interpretation, the compound has also been utilized in behavioral studies to compare the behavior functions of AC- and PI-coupled D1-like receptor activation. For example, SKF 83959 was reported to differ from the AC-coupled SKF 83822 in stimulating the onset of maternal behaviors in rats (Stolzenberg, Zhang, Luskin, Ranker, Bress, & Numan, 2010). In addition, Cools, Lubbers, Van Oosten, and Andringa (2002) found the intra-accumbal injection of SKF 83959 at high doses to antagonize the locomotor activity induced by SKF 81297, an AC-coupled D_1R agonist. According to Cools et al. (2002), the administration of SKF 83959 alone did not have significant effects on locomotor activity. Using the rat model of 6-OHDA lesion, Zhang, Ma, Wang, Chen, and Zhen (2007) reported that SKF 83959 induced contralateral rotations with less severe symptoms of dyskinesia. Moreover, the chronic administration of SKF 83959 was found to decrease the dyskinesia induced by L-DOPA (Zhang et al., 2007). In monkeys, high doses of SKF 83959 have been reported to induce catalepsy (Platt, Rowlett, & Spealman, 2000).

Apart from the studies using reflexive behavioral models as described above, several studies related to cocaine addiction have tested the effects of SKF 83959 on tasks involving conditioned behaviors in monkeys. Platt et al. (2000) found that the pretreatment of SKF 83959

dose-dependently attenuated the cocaine-induced increases in response rates on 10-minutes of fixed-interval (FI) shock termination tasks, and produced rightward shifts in the cocaine dose-response functions on fixed-ratio (FR) cocaine discrimination tasks in squirrel monkeys. The administration of SKF 83959 alone produced cocaine-reversible decreases in the overall response rates at doses that did not induce catalepsy or muscle rigidity (Platt et al., 2000). Similarly, using second-order FR 10 or FR 30 schedules within 10-minute intervals, Platt, Rowlett, and Spealman (2001) found the pretreatment of SKF 83959 to produce dose-related decreases in the response rates on self-administration tasks for cocaine and food pellets respectively. In another study on reinstatement to cocaine priming using the same second-order schedules, Khroyan, Barrett-Larimore, Rowlett, and Spealman (2000) demonstrated that SKF 83959 on its own did not induce reinstatement of responding at the doses tested, while the co-treatment of SKF 83959 with an effective priming dose of cocaine showed dose-dependent inhibition on the reinstatement of responding in the monkeys. From these findings, SKF 83959 appeared to exhibit antagonist-like effects on cocaine-induced behaviors and demonstrated a general suppressive effect on the overall response rates of operant performance.

As the functional role and cellular mechanism of D_1-D_2 receptor heteromers remain unclear, an investigation on the effects of SKF 83959 on operant behaviors is an intriguing attempt to determine the possible behavioral functions of D_1-D_2 receptor heteromer activation.

DA and Schedule-Controlled Behaviors

DA transmission in the brain has been known to possess a critical role in reward- and motivation-mediated behaviors. The design and introduction of the operant chamber by Skinner

(1938), which consisted of a box with at least one lever inside for collecting behavioral responses from subject animals, has been a classic animal model to test the relationship between DA transmission and incentive-motivated behaviors (Salamone & Correa, 2012). In this model, animals are usually deprived of a primary reinforcer such as food or water, in order for them to become motivated to learn and behave according to the schedules of reinforcement to obtain the reward. Different schedules of reinforcement provide means to study the animals' choice behaviors under different external contexts (Morgan, 2010). Specific schedules of reinforcement are characterized by distinct behavioral components that can be used to probe the particular DA-related behavioral function (Sanger & Blackman, 1989).

Two common types of reinforcement schedules are the continuous reinforcement, under which a response is reinforced every time it occurs, and the partial or intermittent reinforcement, when only parts of the responses are reinforced (Chance, 1979). Schedules of reinforcement vary on the basis of frequency ratios or time intervals; ratio schedules are based on the numbers of responses emitted by the subject, while interval schedules are based on responding after the passage of a specified time interval (Schoenfeld, Cumming, & Hearst, 1956; Zeiler, 1977). For example, every n^{th} response is reinforced regularly under FR schedules, whereas the animal's first response after a designated time interval of n seconds is regularly reinforced under FI schedules (Chance, 1979).

Both ratio and interval schedules serve to test the acquisition and performance of operant behaviors in animals, yet the use of interval schedules additionally allows the measurement of their capabilities in timing performance. Interval timing refers to the ability to discriminate and produce temporal durations within the seconds to minutes range (Drew, Fairhurst, Malapani, Horvitz, & Balsam, 2003). Interval timing is also a DA-related function as striatal MSNs have

been proposed to help in the discrimination of temporal durations by detecting the patterns of cortical oscillations from neuronal firing in the network of the nigrostriatal and mesocorticolimbic pathways (Meck et al., 2008). Finally, the option of differential schedules of reinforcement allows researchers to selectively reinforce some desirable aspects of animal behavior in their experimental design. The differential reinforcement of low rate (DRL) that decreases the animals' response rates while maintaining a previously-acquired operant behavior is one of the representative examples of the differential schedules of reinforcement (Chance, 1979).

A main difference between ratio and interval schedules is in the generation of response rates; ratio schedules tend to maintain higher response rates than interval schedules under the same rates of reinforcement, and interval schedules are able to maintain responding when the rates of reinforcement are very low, a characteristic that is not observed in ratio schedules (Baum, 1993). The present proposed study will use interval schedules as behavioral tasks to investigate the DA-related functions of reward-motivated operant behaviors and timing performance, namely the FI 30-s and DRL 10-s schedules of reinforcement. The former presents a reward every 30 seconds after the trial starts, hence the animal eventually learns to start responding as the end of the 30 seconds lapse approaches; the plots of the inter-response time (IRT) of FI schedules are usually scallop-shaped, reflecting an increase in responding as the designated interval approaches. In contrast, the DRL 10-s schedule presents a reward only when the animal makes a response that has been at least 10 seconds from its previous response; any responses within the 10 seconds lapse will reset the countdown. In other words, the animal will learn to withhold from responding during the set interval under DRL schedules in order to gain the reward. Hence the DRL 10-s schedule maintains a lower rate of responding in the animals than

the FI 30-s schedule (Chang, Liao, Lan, & Shen, 2000; Chiang, 2006).

Despite the difference in the generation of response rates, the FI 30-s and DRL 10-s schedules of reinforcement are characterized by different behavioral components and supposed to involve different neural mechanisms. Although both tasks require normal motor abilities for making responses, the FI 30-s schedule probes the animals' abilities in timing over fixed intervals, while the DRL 10-s schedule has more stringent rules for timing over resettable periods. Moreover, the DRL 10-s schedule is characterized by a unique component of behavioral inhibition since the animals need to learn to withhold from responding for a certain length of time.

The Operant Behavioral Pharmacology of DA-Related Drugs

Previous pharmacological studies have utilized various direct or indirect DA agonists and antagonists to test the effects of drug-induced enhancement or blockade in DA transmission on operant behaviors. The respective administration of selective D_1R and D_2R antagonists (SCH 23390 and YM 09151-2) was found to reduce the rates of lever-pressing for food on FR schedules in rats (Rusk & Cooper, 1994). It was generally concluded that the blockade of D_1R and D_2R decreased the hedonic effects of reward on animals' responding (Beninger & Miller, 1998). Similarly, the respective administration of D_1R- and D_2R-selective agonists (SKF 38393 and N-0437) was also found to decrease the operant responding for food under FR schedules of reinforcement (Rusk & Cooper, 1989; Rusk & Cooper, 1988).

In respective to interval-based operant tasks, the administration of DA agonists to rats trained under the peak interval procedure was found to speed up their internal clock, leading to

an underestimation of time intervals; alternatively, the administration of DA antagonists slowed down their internal clock, resulting in overestimated time intervals (Drew et al., 2003). Multiple studies have found the administration of amphetamine-related compounds to shift the peak time of the performance on various DRL schedules, including DRL-15s, DRL-16s, and DRL-36s, to the left (Sanger, Key, & Blackman, 1974; Segal, 1962; Sabol, Richards, Layton, & Seiden, 1995). Similarly, the respective administration of D_1R- and D_2R-selective agonists (SKF 38393 and quinpirole) to rats was observed to result in a leftward shift on the response distributions of peak interval procedures, which consisted of learning trials, when rewards are given at a fixed time interval after cue presentation, and peak trials, when cues are presented for an extended length of time in the absence of rewards (Frederick & Allen, 1996). On FI tasks, amphetamine-related compounds were found to induce an increase in response rates early on during the intervals, which was also suggestive of a faster internal clock (Maricq, Roberts, & Church, 1981). In contrast, the administration of D_1R- and D_2R-selective antagonists (SCH 23390 and eticlopride) induced a rightward trend in the response distributions of peak interval procedures that did not reach statistical significance (Frederick & Allen, 1996). Also using the peak interval procedure, Drew et al. (2003) compared the effects of the D_1R antagonist SCH 23390 and D_2R antagonist haloperidol on two time intervals (12 and 36 sec) of operant performance; the administration of SCH 23390 and haloperidol both decreased the response rates, while haloperidol impaired the timing performance (Drew et al., 2003).

In summary of the above findings, the respective activation and blockade of D_1Rs and D_2Rs both resulted in decreased response rates for food under FR schedules. In contrast, the pharmacological activation of D_1Rs and D_2Rs induced underestimated time intervals and a faster internal clock, whereas the blockade of D_1Rs and D_2Rs resulted in overestimated time intervals

and a slower internal clock. Although the D_1Rs and D_2Rs exert opposite modulation over AC, they do not necessarily affect behaviors oppositely to each other. Hence it may be useful to examine the potential relationship between changes in behavioral performance and cellular signaling in order to better understand the functions of the DARs.

Objective of the Present Study

As previous research mainly examined the functions and neural mechanisms of D_1R and D_2R monomers, the present study aimed to investigate the behavioral and biochemical changes that may be induced by the activation of the recently-identified D_1-D_2 receptor heteromers. The D_1-D_2 receptor heteromers were pharmacologically activated via the administration of SKF 83959, a selective D_1-D_2 receptor heteromer agonist (Neumeyer et al., 2003).

In Experiment 1, the operant performance on the FI 30-s and DRL 10-s schedules of reinforcement were measured in rats under different doses of SKF 83959 administration. As stated earlier, these two interval-based tasks consisted of different behavioral components, whose contrast may provide insights to the particular drug-induced changes in animal behavior. SKF 83959 was intraperitoneally (i.p.) injected at the doses of 0.01, 0.1, and 1.0 mg/kg relative to the vehicle treatment (0 mg/kg) in a within-subjects design to determine the dose effects of SKF 83959 on the respective schedules of operant behaviors. The doses chosen were in reference to Zhang et al. (2005).

In Experiment 2, the brain tissues were collected from the rats after behavioral testing under the effective dose of SKF 83959 to determine if there were any drug-induced brain region-specific changes in the patterns of selected protein expression using Western Blot. To do so, the

rats were divided into two groups and received an i.p. injection of either the vehicle or the most effective dose of SKF 83959 from Experiment 1 in a between-subjects design. The behavioral test session was immediately followed by animal sacrifice and tissue collection. Brain tissues were collected from regions of the mesocorticolimbic system, including the PFC, DS, NAc, and dorsal hippocampus. The levels of expression of the following four proteins in their phosphorylated versus native forms in each of the four selected brain regions were analyzed: 1) CaMKII α, a calcium-dependent protein kinase downstream of D_1-D_2 receptor heteromer activation and NMDAR activation, to be used as an indicator of calcium modulation; 2) PKA, a kinase downstream of the classic AC-coupled D_1R pathway, to be used as an indicator of typical D_1R activation; 3) ERK, a kinase downstream of NMDAR activation that is also indirectly modulated by D_1R activation; 4) CREB, a transcription factor in the cell nucleus that regulates gene expression. Any task-specific or brain region-specific changes in the levels of the proteins selected above were investigated. Also, the statistical correlation between the behavioral indexes and protein levels was conducted to determine the possible trends that underlie the effects of D_1-D_2 heteromer activation via SKF 83959 administration.

In Experiment 3, a separate batch of naive rats was used to test the dose effects of SKF 83959 on locomotor activity using the open field test. The purpose of the locomotor activity test was to investigate whether SKF 83959 might have any debilitating impacts on the rats' motor functions that may potentially impair their performance on the operant tasks.

In Experiment 4, experiments of pharmacological antagonism tested the effects of SCH 23390 and eticlopride pretreatments in antagonizing the effects of SKF 83959 on operant behaviors. SCH 23390 and eticlopride are selective DAR antagonists binding on D_1Rs and D_2Rs respectively.

Together, the present study investigated whether SKF 83959, a putative agonist for D_1-D_2 heteromers, would affect the behavioural performance on two interval-related schedules of reinforcement. Also, the biochemical effects of SKF 83959 on the levels of selected protein expression in the mesocorticolimbic DA system were investigated. Based on a previous behavioral study on SKF 83959, the compound exhibited DA antagonist-like effects on the operant response rates in monkeys (Platt et al., 2000). Hence the administration of SKF 83959 in the present study was hypothesized to decrease the response rates in rats on the operant tasks. It was of interest to find out whether the drug would affect temporal processes in the rats. Based on previous knowledge about the biochemical properties of SKF 83959 (Rashid et al., 2007), it was hypothesized that the drug would not significantly change the levels of PKA. SKF 83959 was hypothesized to exhibit greater impacts on the levels of pCaMKII or CaMKII in the regions encompassing the mesocorticolimbic DA system. It was also of interest to investigate the drug effects on the levels of ERK and CREB in the brain DA terminals, as no such report has been made at this point.

Methods

Subjects

The experiments were conducted using male Wistar rats purchased from Biolasco Taiwan Co., Ltd as subjects. They were housed individually in stainless steel hanging cages in a temperature- (23˚C) and humidity- (60%) controlled colony room under 12 hours of light and dark cycles (lights on from 8am to 8pm). Initially the rats had unrestricted access to water and food until their body weight reached 280-300 g; after which access to water was restricted to 5 minutes per day and behavioral training commenced. During the subsequent training and experimental stages, access to water was maintained at 5 minutes per day, made available to the rats at 30 minutes after the completion of the behavioral procedures. Access to food remained *ad libitum*.

Apparatus

Operant behaviors. Operant responses were measured using six equal operant chambers (MED Associated, St. Albans, VT, USA), in the dimensions of 20 x 25 x 30 cm. The floors of the chambers were composed of 18 stainless steel rods with the diameter of 5 mm, each spaced 11 mm apart; the panels on the sides were made of aluminum, and the front and back walls of clear Plexiglas. The ceiling of the chambers consisted of clear Plexiglas. Each chamber contained a lever, a water-receiving dish connected to an external liquid dispenser, and a house light. The lever was a 4.5 x 2 cm piece of metal positioned 7 cm above the floor and 2 cm from the left corner of the front panel. The liquid dispenser was installed on the outside of each chamber, set to deliver 0.03 to 0.05 ml of tap water per reinforcement to the receiving dish

located at the center of the front panel 2 cm above the floor for each reinforced response. The light source in each chamber was provided by a light bulb of 2.5 cm diameter, located 12 cm above the floor and 2 cm from the left corner of the front panel, right above the lever. The chambers were enclosed in 60 x 40 x 58 cm plywood boxes with fans installed on the top right-hand corners for ventilation and controlled background noise.

The operant chambers were connected to computers for automatic control via the printer port and PCI interface card for input and output. The computer program for setting up the different reinforcement schedules and data recording was written in Visual Basic (version 6.0 for Windows 98; Microsoft) (Liao & Cheng, 2007). During the FI task, the computer program collected three behavioral indexes, which included the inter-response time (IRT; the lapse of time between responses), the number of total responses, the number of reinforced responses, and the post-reinforcement pause (PRP; the lapse of time between a reinforced response and its next response). During the DRL task, the program similarly collected the IRT, the number of total responses, the number of reinforced and non-reinforced responses; additionally, the program recorded the number of burst responses (responses within 3 seconds from previous responding), and calculated the peak time (the mean value of IRTs that fall within the 4 consecutive 1-sec bins containing maximum response frequencies), the peak rate (the sum of response frequencies during the 4 bins divided by 4), and the modified response efficiency (MRE; the ratio between the number of reinforced responses and the number of total responses minus the number of burst responses) (Liao & Cheng, 2007).

Locomotor activity. The open field locomotor activity test was conducted in an assembly of four identical black acrylic boxes (45 x 45 x 36 cm each). A charge coupled device (CCD) camera was installed above the center of the assembly at 52 cm from the ground. The

camera was connected to a desktop computer, which recorded the distance travelled by the rats as well as their average and maximum speed.

Training of FI and DRL Behaviors

The water-deprived rats started with the magazine training, during which they learned to associate water with the metal receiving dish. Then the rats continued on to three daily 30-minute sessions of shaping on the FR 1 schedule of reinforcement, from which the rats learned to associate lever presses with the appearance of the reinforcer (water) on the receiving dish. All of the rats were expected to make at least 65 lever presses in a 30 min training session to meet the criterion of this stage. The rats were then randomly assigned into separate groups for training under either the FI 30-s (n = 12) or DRL 10-s (n = 12) schedules of reinforcement.

Rats in the FI group began daily training under one-hour sessions of the FI 10-s schedule for 10 days until their average number of total responses reached 900. Then the rats continued with daily 30-minute training sessions under FI 30-s for 25 days until the average number of total responses reached 600 before the commencement of pharmacological test sessions. In contrast, rats in the DRL group were trained by hourly sessions of the DRL 10-s schedule for 10 days followed by daily 30-minute training sessions for 25 days; the testing of drug-induced performance began when their average number of total responses reached 200. Over the course of the operant training, stable baseline performance on both tasks were indicated by the criterion of less than 10% of variation on average response rates for three consecutive days.

Drugs

SKF 83959 hydrobromide (Tocris Bioscience) was dissolved in the vehicle containing 10% ethanol. SCH 23390 hydrochloride (RBI, Research Biochemicals Inc.) and eticlopride hydrochloride (Tocris Bioscience) were dissolved in 0.9% physiological saline. All drugs were i.p. injected at the volume of 1 ml/kg of body weight.

Western Blot

The collected brain tissues were added with protease inhibitor, phosphatase inhibitor, and lysis buffer for homogenization, centrifugation, and dilution with lysis buffer. The lysates were tested for protein concentrations, prepared into samples of 1 μg/μl, and boiled for 10 minutes at 90°C before loading for Western blot. The sample lysates (20 μl) were loaded onto 10% SDS polyacrylamide gel for separation by electrophoresis, and they were later transferred onto a polyvinylidene fluoride (PVDF) membrane via electroblotting. The sections of the membrane that contained the proteins of interest (according to molecular weight) were cut out into a strip and immersed in 2% BSA (bovine serum albumin) for 1 hour of blocking at 40 rpm of autoshaking under room temperature. The membrane was then incubated with the primary antibody (diluted 1:2000) on an autoshaker overnight at 4 °C. The primary antibodies included the rabbit anti-pCaMKII (Upstate), mouse anti-CaMKII (Millipore), rabbit anti-pERK1/2 (Cell Signaling), rabbit anti-ERK1/2 (Cell Signaling), rabbit anti-PKA (Upstate), mouse anti-pCREB (Millipore), and rabbit anti-CREB (Millipore). On the following day, the membrane was washed three times (for 5, 10, 10 minutes) in 0.1% TBST (Tris-buffered saline, 0.1% Tween 20) and incubated with the secondary antibody (1:4000, either anti-mouse or anti-rabbit IgG conjugated to horseradish peroxidase (HRP)) on a 40 rpm autoshaker for 1 hour under room temperature.

Then the membrane was washed again for three times in 0.1% TBST before detection by chemiluminescent reaction with Immobilon Western chemilminescent HRP substrate (Millipore). The primary antibody for actin was used as an internal control and followed the same protocols. The protein bands were quantified by ImageJ (version 1.47, National Institutes of Health, USA).

Procedures

Experiment 1: The dose effects of SKF 83959 on FI 30-s and DRL 10-s performance. When the rats were trained to stable baseline performance on the operant tasks, they were tested for the dose effects of SKF 83959. The dose treatments of SKF 83959 (0, 0.01, 0.1, and 1.0 mg/kg) were given via i.p. administration in a within-subjects design for the DRL 10-s (n = 11) and FI 30-s (n = 12) groups on four consecutive days. The drug administration was given 30 minutes prior to the behavioral session. After injection, the rat was kept in a holding cage, located in the behavior test room, for 30 minutes until it was placed into the operant chamber. A 30-minutes session under the FI 30-s or DRL 10-s schedule of reinforcement was conducted in this dose-effect test.

Experiment 2: The effects of SKF 83959 on operant performance and levels of selected protein expression in mesocorticolimbic DA terminals. The rats from Experiment 1 underwent six days of retraining on their respective schedules of reinforcement until the performance returned to stable baseline. The rats within the FI or DRL group were then divided to receive either the vehicle (n = 6 in the FI 30-s groups and n = 5 in the DRL 10-s group) or the most effective dose of SKF 83959 (n = 6 for each schedule) in a between-subjects design. The rats in the FI 30-s group that made less than 3 lever presses in Experiment 1 (#15, 17, 20, 21, 23)

were randomly distributed to the vehicle group (#17, 21, 23) and the drug treatment group (#15, 20). The rat in the DRL 10-s group that made no lever presses in Experiment 1 (#3) was placed into the vehicle group in Experiment 2. Using the aforementioned subject assignment, there were no significant differences in the baseline numbers of total responses between groups before drug injections in Experiment 2. On the FI 30-s schedule, the vehicle group made 562 ± 98 responses, and the experimental group made 565 ± 53 responses ($F = 0.0008$, $p > 0.05$). On the DRL 10-s schedule, the vehicle group made 195 ± 9 responses while the experimental group made 193 ± 21 responses ($F = 0.0099$, $p > 0.05$). The procedures of the drug test on operant behaviors were the same as the description in Experiment 1. Following the drug-treated behavior test, the rats were decapitated. Tissues were collected from the specified brain regions including the PFC, DS, NAc, and dorsal hippocampus (Figure 1). The collected brain tissues were treated with liquid nitrogen, and stored in a -80°C freezer until preparation for Western blot analysis.

Experiment 3: The effects of SKF 83959 on locomotor activity. A separate batch of naive rats ($n = 18$) was divided into 3 groups for either 0, 0.5, or 1.0 mg/kg of i.p. SKF 83959 injection ($n = 6$ each). After the respective drug injection, rats were placed in their holding cages for 30 min before being placed into the open field boxes for a 30-min locomotor activity test. The average travel speed and total travel distance were recorded in 5-min intervals.

Experiment 4: The effects of SCH 23390 and eticlopride pretreatment on SKF 83959-induced operant performance. The respective effects of pretreating SCH 23390, eticlopride, and their combination on SKF 83959-induced operant behaviors were tested by giving two (or three) i.p. drug injections during each session. The first injection was given 60 min before the behavioral test, and the second injection was given 30 min before the behavioral

test. On the first test day, all rats were subjected to control treatments (the double injection of saline-vehicle). After a day of retraining, the rats went on to receive the drug treatments of saline-SKF 83959, DAR antagonist-saline, and DAR antagonist-SKF 83959 in the orders of a Latin square on test sessions each spaced apart by two days of retrain. SKF 83959 was injected at 1.0 mg/kg, which was the effective dose from Experiment 1 and Experiment 2. SCH 23390 and eticlopride were both tested at 0.02 and 0.06 mg/kg, based on previous studies from this lab (Cheng & Liao, 2007) and others (Fowler & Liou, 1998; Schindler & Carmona, 2002).

SCH 23390. The rats from Experiment 3 started water deprivation two weeks after the locomotor activity test, and they were divided into two groups to receive operant training under either the FI 30-s (n = 9) or DRL 10-s (n = 9) schedules of reinforcement, in the procedures as described earlier. After reaching the stable baseline, the rats were subjected to the tests with 0.02 mg/kg SCH 23390 pretreatments. The rats were retrained for three days, after which they were subjected to testing with 0.06 mg/kg SCH 23390 pretreatments.

Eticlopride. A separate batch of naïve rats (n = 18) were divided into the FI 30-s and DRL 10-s groups for operant training as described above. When they reached stable baseline performance, the rats were subjected to operant testing with 0.02 mg/kg eticlopride pretreatments. After three days of retraining, they were again subjected to testing with 0.06 mg/kg eticlopride pretreatments.

The combination of SCH 23390 and eticlopride. After the eticlopride treatments, the rats were retrained for three days before being subjected to the combined pretreatments of 0.02 mg/kg SCH 23390 and 0.02 mg/kg eticlopride. The control treatments on the first day were a

triple injection of saline-saline-vehicle. On experimental sessions, the double injections of SCH 23390 and eticlopride were given in alternating orders.

Statistical Analysis

The data are presented in mean ± the standard error of the mean (SEM), and analyzed with ANOVA using Statistica (version 7.1, StatSoft). *Post-hoc* comparisons were conducted using Tukey's honestly significant difference (HSD) test with a significance level of $p < 0.05$. In the exceptions of cases where the ANOVA yielded significant results and the Tukey's HSD did not, the least significant difference (LSD) test was used to account for possible over-corrections by Tukey's HSD when sample sizes were not large enough. Bivariate correlations between behavioral measures and protein results were conducted with SPSS (version 16.0, SPSS Inc.).

It should be noted that some rats appeared to completely cease operant responding upon the administration of 1.0 mg/kg SKF 83959 (see Table 1). Cases of such low (number of total responses less than five) or zero responses were still included in the 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 the analysis of response-based indexes, while the group mean was substituted in these cases of missing data on the other indexes: PRP on the FI task, peak time, peak rate, and MRE on the DRL task.

For some of the rats whose levels of protein expression obviously deviated from the group mean, their protein data were considered as outliers and removed from the analysis of that particular protein. This did not exclude them from the behavioral analysis as the deviation was attributed to possible human error during the process of Western blotting.

Results

Experiment 1: The Dose Effects of SKF 83959 on FI 30-s and DRL 10-s Performance

The FI 30-s task. As illustrated in Figure 2, the rats trained under the FI 30-s schedule exhibited a dose-dependent decrease in response rates on the IRT curve of a 30-s interval as they received higher doses of SKF 83959 injections. One-way ANOVA showed that drug dose had significant effects on the numbers of total responses, $F(3, 33) = 32.95$, $p < 0.001$, reinforced responses, $F(3, 33) = 22.51$, $p < 0.001$, and the duration of PRP, $F(3, 33) = 26.11$, $p < 0.001$. As indicated by Tukey's HSD *post-hoc* test, the rats emitted significantly fewer numbers of total responses when they were injected with SKF 83959 at the medium dose (0.1 mg/kg; $p < 0.01$) and the high dose (1.0 mg/kg; $p < 0.001$) (Figure 3A). The rats also showed significantly fewer numbers of reinforced responses upon high dose SKF 83959 administration (1.0 mg/kg; $p < 0.001$; Figure 3B); their durations of PRP were significantly increased after injections with medium and high dose SKF 83959, $p < 0.01$ and $p < 0.001$ respectively (Figure 3C). As noted in Table 1, three of the twelve rats tested had completely ceased to respond on the lever during the test session upon 1.0 mg/kg SKF 83959 administrations, while an additional number of two rats emitted equal to or less than 2 lever presses during the session after high dose injection.

The DRL 10-s task. The rats trained under the DRL 10-s schedule similarly showed a dose-dependent decrease in response rates on the IRT curve as they were injected with higher doses of SKF 83959, while the peak time remained relatively in place (Figure 4). One-way ANOVA found the drug dose to have significant effects on three of the four response-based indexes, including the numbers of total responses, $F(3, 30) = 4.53$, $p < 0.01$, reinforced responses, $F(3, 30) = 3.49$, $p < 0.05$, and non-reinforced responses, $F(3, 30) = 3.49$, $p < 0.05$. Tukey's HSD

post-hoc test showed that the total responses were significantly decreased when the rats were treated with high dose SKF 83959, $p < 0.05$ (Figure 5A). *Post-hoc* by LSD also found significantly reduced numbers of reinforced responses, $p < 0.05$ (Figure 5B) and non-reinforced responses, $p < 0.05$ (Figure 5C) under high dose SKF 83959 administration. The number of burst responses was not affected by SKF 83959, $p > 0.05$ (Figure 5D). In terms of the other DRL indexes, the peak rate was significantly affected by the doses of SKF 83959, $F(3, 30) = 6.58$, $p < 0.01$. Tukey's HSD showed significantly decreased peak rate under high dose SKF 83959 injection, $p < 0.01$ (Figure 6A). The peak time and MRE were unaffected by SKF 83959 administrations (Figure 6B & 6C respectively). During the DRL test, one of the eleven rats tested emitted no responses upon high dose SKF 83959 injection.

Experiment 2: The Effects of SKF 83959 on Operant Performance and Levels of Selected Protein Expression in Mesocorticolimbic DA Terminals

Operant performance. The behavioral effects of 1.0 mg/kg SKF 83959 replicated the findings of Experiment 1 to a great extent.

FI 30-s task. As shown in Figure 7, the administration of 1.0 mg/kg SKF 83959 reduced the response rates on the IRT curve. In comparison to the vehicle group, the SKF 83959-treated rats showed significantly decreased numbers of total responses, $F(1, 10) = 23.49$, $p < 0.001$ (Figure 8A) and reinforced responses, $F(1, 10) = 16.12$, $p < 0.01$ (Figure 8B). The drug-treated rats exhibited significantly increased durations of PRP, $F(1, 10) = 18.53$, $p < 0.01$ (Figure 8C). One of the six rats in the experimental group emitted no responses upon SKF 83959 injection.

DRL 10-s task. Similarly, the administration of 1.0 mg/kg SKF 83959 also reduced the frequency of lever presses on the IRT curve (Figure 9). The drug-treated rats showed

significantly reduced numbers of total responses, $F(1, 9) = 7.51$, $p < 0.05$ (Figure 10A), non-reinforced responses, $F(1, 9) = 7.95$, $p < 0.05$ (Figure 10C), and burst responses, $F(1, 9) = 11.83$, $p < 0.01$ (Figure 10D). Although the drug-treated rats also exhibited a decreasing trend in the numbers of reinforced responses, this effect did not reach statistical significance (Figure 10B). In terms of the other indexes on the DRL task, SKF 83959-treated rats exhibited significantly lowered peak rates, $F(1, 9) = 6.00$, $p < 0.05$ (Figure 11A). The peak time was unaffected by drug treatment (Figure 11B), and the calculated value of the MRE ratio was significantly higher relative to the vehicle group, $F(1, 9) = 6.09$, $p < 0.05$ (Figure 11C). One of the six rats in the experimental group did not respond upon SKF 83959 injection.

Protein expression.

CaMKII. Figure 12 illustrates the biochemical effects of SKF 83959 on the expression of CaMKII proteins in the four specified brain regions of rats from the FI 30-s and DRL 10-s groups respectively. The drug-treated rats in the FI 30-s group were found to possess a significant decrease in their levels of phosphorylated CaMKII (pCaMKII) expression in the NAc relative to the vehicle-treated rats, $F(1, 10) = 8.14$, $p < 0.05$, while no significant changes were found in the PFC, DS, or hippocampus (Figure 12A). Alternatively, the drug-treated rats exhibited a significant increase in the levels of native CaMKII expression relative to the vehicle group in the hippocampus, $F(1, 10) = 13.29$, $p < 0.01$, which was not observed in the PFC, DS, or NAc (Figure 12B). The ratio between the levels of pCaMKII and native CaMKII was calculated to examine the proportions of protein activation in the various brain regions. Such analysis revealed a significant decrease in the pCaMKII/CaMKII ratio only in the hippocampus of drug-treated rats relative to the vehicle-treated rats, $F(1, 10) = 9.14$, $p < 0.05$ (Figure 12C).

In Figure 12D, the SKF 83959-treated rats in the DRL 10-s group showed a significant

decrease in the levels of pCaMKII expression in the DS relative to the vehicle group, $F(1, 7) = 5.75$, $p < 0.05$, and no statistically significant changes were observed between the drug- and vehicle-treated rats in the PFC, NAc, and hippocampus. In respective to the levels of CaMKII expression, the drug-treated rats exhibited a significant decrease in their levels of CaMKII in the DS, $F(1, 8) = 10.09$, $p < 0.05$, and hippocampus, $F(1, 8) = 5.72$, $p < 0.05$, relative to the vehicle-treated rats, and not in the PFC or NAc (Figure 12E). The comparison of the ratio between pCaMKII and CaMKII levels did not reveal statistically significant differences between the drug- and vehicle-treated rats in the brain regions tested (Figure 12F).

ERK. The analysis of ERK expression has been divided into that of ERK1 expression, ERK2 expression, and the sum of both (total ERK expression). In respective to the levels of phosphorylated ERK (pERK) expression in the FI 30-s group, there were no significant differences between treatments across the brain regions tested (Figure 13A). In a closer examination at the expression of pERK1 and pERK2, a significant increase in the levels of pERK1 in the DS, $F(1, 9) = 7.69$, $p < 0.05$, and a significant decrease in the levels of pERK2 in the hippocampus, $F(1, 7) = 6.92$, $p < 0.05$, were observed in the drug-treated rats relative to the vehicle group (Figure 14A and Figure 15A). The changes in pERK1 or pERK2 levels between treatments in the other brain regions tested did not reach statistical significance, despite there was also a trend of decrease in the expression of pERK1 in the hippocampus. Next, the level of total native ERK expression in the FI 30-s group was significantly increased only in the DS of drug-treated rats relative to vehicle, $F(1, 10) = 54.69$, $p < 0.001$ (Figure 13B). The individual inspection of ERK1 levels showed a significant increase in the PFC, $F(1, 8) = 12.67$, $p < 0.01$, and DS, $F(1, 9) = 22.57$, $p < 0.01$, of drug-treated rats relative to vehicle, and not in the NAc or hippocampus (Figure 14B). The examination of ERK2 expression revealed significant increases

in the DS, $F(1, 8) = 59.00$, $p < 0.001$, and hippocampus, $F(1, 7) = 9.00$, $p < 0.05$, of drug-treated rats relative to vehicle, but not in the PFC or NAc (Figure 15B). Finally, no significant difference was yielded in the ratios of total pERK and total ERK between treatments on any tested brain areas, despite a minor trend of decrease in the drug-treated rats relative to the vehicle from visual examination (Figure 13C). Alternatively, the inspection of pERK1 to ERK1 ratios only showed a significant decrease in the hippocampus of the drug-treated rats, $F(1, 7) = 11.13$, $p < 0.05$ (Figure 14C). The analyses of pERK2 to ERK2 ratios revealed significant decreases in the DS, $F(1, 8) = 9.43$, $p < 0.05$, and hippocampus, $F(1, 7) = 11.13$, $p < 0.05$, of drug-treated rats relative to vehicle, but not in the PFC and NAc (Figure 15C).

In rats from the DRL 10-s group, there were no significant differences in the expression of total pERK across brain regions between treatments (Figure 13D). The closer examination of pERK1 expression showed significant decreases in the PFC, $F(1, 7) = 9.10$, $p < 0.05$, DS, $F(1, 7) = 9.62$, $p < 0.05$, and NAc, $F(1, 7) = 6.25$, $p < 0.05$ of the drug-treated rats relative to vehicle. The trend of decrease in the hippocampus was not statistically confirmed (Figure 14D). In respective to the expression of pERK2, there was a significant decrease in the hippocampus of the drug-treated rats relative to vehicle, $F(1, 7) = 6.92$, $p < 0.05$, and there were no significant differences in the PFC, DS, or NAc between treatments (Fiigure 15D). The expression of total native ERK was not significantly affected by SKF 83959 administrations across the brain regions tested (Figure 13E). However, the individual examination of native ERK1 and ERK2 expression revealed a significantly lower ERK1 level in the hippocampus, $F(1, 7) = 10.06$, $p < 0.05$, and a significantly lower ERK2 level in the DS, $F(1, 7) = 11.27$, $p < 0.05$, of drug-treated rats relative to vehicle. The expression of native ERK1 and ERK2 were not significantly affected between treatments in the other brain regions tested (Figure 14E and Figure 15E). In the analysis of pERK

to ERK ratios, there were no significant differences in the total value of pERK over ERK (Figure 13F), pERK1 over ERK1 (Figure 14F), and pERK2 over ERK2 (Figure 15F) between treatments in the DRL 10-s group.

PKA. As demonstrated in Figure 16A, the rats in the FI 30-s group that received SKF 83959 injections exhibited a significant decrease in the levels of PKA expression in the NAc relative to those that received vehicle injections, $F(1, 10) = 6.65$, $p < 0.05$. The changes in PKA levels between the drug- and vehicle-treated rats did not reach statistical significance in the other three brain regions tested. Alternatively, the levels of PKA expression were significantly lower in the PFC, $F(1, 9) = 5.58$, $p < 0.05$, and significantly higher in the NAc, $F(1, 7) = 8.20$, $p < 0.05$, of the drug-treated rats in the DRL 10-s group in comparison to the vehicle-treated rats (Figure 16B). No significant differences were found between the rats in the DS and hippocampus.

CREB. Figure 17 illustrates the expression of CREB between drug treatments in the FI 30-s and DRL 10-s groups respectively. The drug-treated rats in the FI 30-s group possessed significantly lower levels of phosphorylated CREB (pCREB) in the NAc, $F(1, 10) = 6.60$, $p < 0.05$, and hippocampus, $F(1, 10) = 19.08$, $p < 0.01$, relative to the vehicle-treated rats (Figure 17A). There were no significant differences in the levels of pCREB between treatments in the PFC and DS in the FI 30-s group. In terms of native CREB expression, the SKF 83959-treated rats exhibited significantly lower levels of CREB in the NAc, $F(1, 10) = 7.76$, $p < 0.05$, and hippocampus, $F(1, 10) = 6.97$, $p < 0.05$, relative to vehicle (Figure 17B). No significant differences in CREB expression were found between treatments in the PFC and DS. The examination of pCREB to CREB ratios in the FI 30-s group showed a significant reduction only in the hippocampus of drug-treated rats relative to vehicle, $F(1, 10) = 5.22$, $p < 0.05$ (Figure 17C).

In the DRL 10-s group, a significantly higher level of pCREB expression was observed in the NAc, $F(1, 9) = 14.03$, $p < 0.01$, of the drug-treated rats relative to vehicle, but not in the PFC, DS, or hippocampus (Figure 17D). In respective to the levels of native CREB, there were no significant differences between treatments across the brain regions tested (Figure 17E). Finally the analysis of the ratios between pCREB and CREB expression revealed significant increases in the PFC, $F(1, 9) = 12.51$, $p < 0.01$, and NAc, $F(1, 9) = 6.75$, $p < 0.05$, of the drug-treated rats relative to vehicle. Neither of the DS nor the hippocampus of the DRL 10-s group showed such a difference between treatments (Figure 17F).

Correlation. On the behavioral level, the administration of SKF 83959 was found to be negatively correlated with the numbers of total responses on both of the FI 30-s, $r(10) = -0.838$, $p < 0.01$, and DRL 10-s, $r(9) = -0.674$, $p < 0.05$, tasks. On the biochemical level, correlation analyses were conducted between the levels of the phosphorylated proteins 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.

Prefrontal cortex. A positive relationship between the levels of pCaMKII and PKA was found on both of the FI 30-s, $r(10) = 0.792$, $p < 0.01$ (Table 2) and DRL 10-s , $r(9) = 0.766$, $p < 0.01$ (Table 3) tasks in the PFC. In addition to the correlation between proteins, a negative relationship was found between SKF 83959 administration and PKA expression in the PFC of the DRL 10-s group, $r(9) = -0.617$, $p < 0.05$.

Dorsal striatum. Under the FI 30-s schedule of reinforcement, the levels of pCaMKII, pCREB, and PKA were found to be positively correlated with each other in the DS (Table 4). In contrast, under the DRL 10-s schedule, the levels of pCaMKII was found to be positively

correlated with pERK, $r(9) = 0.760$, $p < 0.01$, and pCREB, $r(9) = 0.725$, $p < 0.05$ in the DS (Table 5).

Nucleus accumbens. In the FI 30-s group, the administration of SKF 83959 was found to be negatively correlated with the expression of pCaMKII, $r(10) = -0.670$, $p < 0.05$, pCREB, $r(10) = -0.631$, $p < 0.05$, and PKA, $r(10) = -0.624$, $p < 0.05$, in the NAc. Of which the levels of pCaMKII and pCREB were positively correlated with each other ($r(10) = 0.690$, $p < 0.05$), as well as with the number of total responses, $r(10) = 0.640$, $p < 0.05$, $r(10) = 0.634$, $p < 0.05$, respectively (Table 6). In the DRL 10-s group, SKF 83959 administration was positively correlated with pCREB expression, $r(9) = 0.780$, $p < 0.01$. The levels of pCaMKII, pCREB, and PKA were positively correlated with each other in the NAc (Table 7).

Dorsal hippocampus. In the FI 30-s group, the administration of SKF 83959 was negatively correlated with the level of pCREB in the hippocampus, $r(10) = -0.810$, $p < 0.01$, while the expression of pCREB was found to be positively correlated with the number of total responses, $r(10) = 0.683$, $p < 0.05$ (Table 8). In contrast, no significant correlations were found between protein levels in the hippocampus of the DRL 10-s group.

Experiment 3: The Effects of SKF 83959 on Locomotior Activity

Two-way ANOVA was performed to determine the effects of drug dose and time intervals on locomotor activity. One rat in the 0.5 mg/kg SKF 83959 group was removed from analysis because it consistently jumped out of the open field box and did not finish the test.

Distance. As shown in Figure 18A, the amount of travelled distance was significantly affected by the elapse of time intervals, $F(5, 70) = 22.16$, $p < 0.001$. Neither the doses main effect nor the interaction test was significant. *Post-hoc* analysis has found significant differences

in travelled distance between that during interval 1 and those during intervals 2 to 6, $p < 0.001$, between that of interval 2 and interval 5, $p < 0.05$, and between those during intervals 2, 3, 4, and interval 6, $p < 0.01$. Overall there was a general decrease in travelled distance as time intervals passed.

Speed. As illustrated in Figure 18B, the travelled speed also significantly affected by the factor of time intervals, $F(5, 70) = 22.15$, $p < 0.001$. The doses of SKF 83959 and the interaction between intervals and drug dose did not have significant effects on travelled speed. *Post-hoc* analysis found significant differences between the speed during interval 1 and those during intervals 2 to 6, $p < 0.001$, between the speed during interval 2 and interval 5, $p < 0.05$, and between the speed during intervals 2, 3, 4, and interval 6, $p < 0.01$. The changes in travelled speed over the 30 minutes test session also exhibited a general pattern of decrease over time.

Experiment 4: The Effects of SCH 23390 and Eticlopride Pretreatment on SKF 83959-Induced Operant Performance

SCH 23390 pretreatment.

FI 30-s performance. Figure 19 illustrates the effects of SCH 23390 treatment alone and before SKF 83959 administration on the IRT curve of FI 30-s performance at two different doses. One of the nine rats was excluded from analysis due to unnoticed equipment errors that have interrupted with proper operant training. The treatment of 0.02 mg/kg SCH 23390 alone did not greatly alter the typical scallop-shaped FI response curve relative to the saline-vehicle treatment, whereas the pretreatment of 0.02 mg/kg SCH 23390 before SKF 83959 injection resulted in decreased responding in a similar manner as the treatment of 1.0 mg/kg SKF 83959 alone (Figure 19A). In contrast, when 0.06 mg/kg SCH 23390 was treated alone or pretreated with

SKF 83959, the response rates on the FI 30-s IRT were decreased in a pattern similar to that of the treatment of SKF 83959 alone (Figure 19B).

Figure 20 displays the behavioral results from the FI 30-s test in terms of the specific indexes under SCH 23390 treatment at two doses. From the tests of 0.02 mg/kg SCH 23390 administrations, one-way ANOVA showed that there were significant differences across the drug treatments on the numbers of total responses, $F(3, 21) = 8.06$, $p < 0.001$, reinforced responses, $F(3, 21) = 7.23$, $p < 0.001$, and PRP, $F(3, 21) = 9.66$, $p < 0.001$. *Post-hoc* analysis by Tukey's HSD showed that when rats were treated with 1.0 mg/kg SKF 83959 alone, they exhibited significantly reduced numbers of total responses, $p < 0.01$ (Figure 20A) and reinforced responses, $p < 0.05$ (Figure 20B). The administration of 0.02 mg/kg SCH 23390 alone did not have significant effects on these two response-based indexes. The pretreatment of 0.02 mg/kg SCH 23390 with 1.0 mg/kg SKF 83959 similarly reduced the numbers of total responses, $p < 0.01$, and reinforced responses, $p < 0.01$, as the treatment of SKF 83959 alone. In addition, the rats that were given the combined treatment of 0.02 mg/kg SCH 23390 and SKF 83959 showed a significant increase in the PRP, $p < 0.001$. The individual treatments of SKF 83959 and SCH 23390 did not produce significant effects on the PRP (Figure 20C).

At the higher dose of SCH 23390 administrations (0.06 mg/kg), one-way ANOVA has found significant differences in the numbers of total responses, $F(3, 21) = 6.18$, $p < 0.01$, and reinforced responses, $F(3, 21) = 7.10$, $p < 0.01$, across treatment conditions. Tukey's *post-hoc* test revealed that when rats were treated with 1.0 mg/kg SKF 83959 alone, they exhibited significantly decreased total responses relative to vehicle, $p < 0.05$, (Figure 20D). This effect was not observed under the treatment of 0.06 mg/kg SCH 23390 alone. The pretreatment of high dose SCH 23390 with SKF 83959 significantly reduced the numbers of total resposnes, $p < 0.01$, in a

similar pattern as that under SKF 83959 alone. The individual administrations of 1.0 mg/kg SKF 83959 and 0.06 mg/kg SCH 23390 did not have statistically significant effects on the numbers of reinforced responses, although the rats under SKF 83959 injections did display a trend of decrease (Figure 20E). Alternatively, the rats under the combined treatment of 0.06 mg/kg SCH 23390 and SKF 83959 exhibited significantly reduced numbers of reinforced responses, $p < 0.01$. One-way ANOVA did not find significant differences across treatment conditions on the index of PRP (Figure 20F).

DRL 10-s performance. Figure 21A and Figure 21B show the effects of SCH 23390 alone and pretreated with SKF 83959 on the IRT curve of DRL 10-s performance at 0.02 mg/kg and 0.06 mg/kg respectively. As shown in both figures, the drug treatments did not shift the peak time. In Figure 21A, the effects of 0.02 mg/kg SCH 23390 alone on the DRL 10-s IRT curve were almost the same as that of vehicle treatments, whereas in Figure 21B, the treatment of 0.06 mg/kg SCH 23390 alone showed a minor effect in reducing the response rate. The pretreatments of neither doses of SCH 23390 with SKF 83959 were found to exhibit reversal effects on the SKF 83959-induced declines in response rate.

Figure 22 and Figure 23 illustrate the quantitative analyses of the behavioral data on the DRL 10-s tests. In respective to the response-based indexes, one-way ANOVA indicated the presence of significant differences across the 0.02 mg/kg SCH 23390 treatment conditions on the numbers of total responses, $F(3, 24) = 19.25$, $p < 0.001$, reinforced responses, $F(3, 24) = 7.20$, $p < 0.01$, non-reinforced responses, $F(3, 24) = 13.82$, $p < 0.001$, and burst responses, $F(3, 24) = 4.13$, $p < 0.05$). *Post-hoc* analysis revealed that when rats were administered with 1.0 mg/kg SKF 83959 alone, they exhibited significant reductions in the numbers of total responses, $p < 0.001$ (Figure 22A), reinforced responses, $p < 0.05$ (Figure 22B), non-reinforced responses, $p < 0.01$

(Figure 22C), and burst responses, $p < 0.05$ (Figure 22D). The administration of 0.02 mg/kg SCH 23390 alone did not have significant effects on any of these four response-based indexes relative to the vehicle treatment. And the pretreatment of 0.02 mg/kg SCH 23390 with SKF 83959 yielded significant reductions in the numbers of total responses, $p < 0.001$, reinforced responses, $p < 0.05$, non-reinforced responses, $p < 0.001$, and burst responses, $p < 0.05$, producing almost the same effects as the treatments of SKF 83959 alone. On the other DRL 10-s indexes, one-way ANOVA indicated significant differences across treatment conditions on the peak rate, $F(3, 24) = 13.10$, $p < 0.001$, and MRE, $F(3, 24) = 7.76$, $p < 0.001$. No significant differences were found across the treatment conditions on the peak time (Figure 23B). As shown in Figure 23A, *post-hoc* tests found the rats to exhibit significantly reduced peak rates relative to vehicle under the treatments of SKF 83959 alone, $p < 0.01$. The administration of 0.02 mg/kg SCH 23390 alone did not have significant effects on the peak rate. The pretreatment of 0.02 mg/kg SCH 23390 with SKF 83959 also induced a decline in peak rates, $p < 0.001$. In respective to the MRE ratio, the rats exhibited a significantly increased MRE when they were treated with SKF 83959 alone, $p < 0.01$. No significant differences in MRE were found under the treatment of 0.02 mg/kg SCH 23390 alone or the combined treatments of SCH 23390 and SKF 83959 (Figure 23C).

The results of 0.06 mg/kg SCH 23390 treatments closely resembled the findings from administering the lower drug dose, with the exception that 0.06 mg/kg SCH 23390 on its own appeared to have some disruptive effects on response rates (Figure 21B). One-way ANOVA indicated significant differences across treatment conditions in the numbers of total responses, $F(3, 24) = 12.77$, $p < 0.001$, reinforced responses, $F(3, 24) = 6.79$, $p < 0.01$, non-reinforced responses, $F(3, 24) = 11.15$ $p < 0.001$, and burst responses, $F(3, 24) = 8.79$, $p < 0.001$. As

shown in Figure 22E through Figure 22H, Tukey's HSD revealed that under the injection of SKF 83959 alone, the rats performed with significant reductions in the numbers of total responses, $p < 0.01$, non-reinforced responses, $p < 0.001$, and burst responses, $p < 0.01$. Although the injections of 0.06 mg/kg SCH 23390 alone did not have significant effects on the response-based indexes, they produced decreases in the numbers of total responses, non-reinforced responses, and burst responses to a greater extent than what was observed from 0.02 mg/kg SCH 23390 injections. Under the injections of SKF 83959 with 0.06 mg/kg SCH 23390 pretreatment, the rats exhibited significantly decreased numbers of total responses, $p < 0.001$, reinforced responses, $p < 0.01$, non-reinforced responses, $p < 0.001$, and burst responses, $p < 0.001$. One-way ANOVA detected significant differences in peak rate, $F(3, 24) = 7.39$, $p < 0.01$, and MRE, $F(3, 24) = 3.38$, $p < 0.05$, across treatment conditions, whereas no significant differences in peak time were found (Figure 23E). As shown in Figure 23D, *post-hoc* tests indicated that the rats performed with significant reductions in peak rate under the treatments of SKF 83959 alone, $p < 0.01$, and SKF 83959 pretreated with 0.06 mg/kg SCH 23390, $p < 0.01$, but not under the treatment of 0.06 mg/kg SCH 23390 alone. In Figure 23F, the rats performed with a significantly increased MRE ratio under the combined treatment of SKF 83959 with high dose SCH 23390, $p < 0.05$, but not under the sole treatments of SKF 83959 or SCH 23390. While the administration of 0.06 mg/kg SCH 23390 alone did not produce significant effects on the peak rate, peak time, or MRE, it appeared to decrease the DRL 10-s peak rate to a greater extent than the treatment of 0.02 mg/kg SCH 23390 alone.

Eticlopride pretreatment.

FI 30-s performance. Figure 24A and Figure 24B illustrate the effects of 0.02 mg/kg and 0.06 mg/kg eticlopride on the IRT curve of FI 30-s performance respectively. The treatment of

0.02 mg/kg eticlopride alone resulted in a curve very similar to that of vehicle treatment, whereas the treatments of 1.0 mg/kg SKF 83959 alone and SKF 83959 with low dose eticlopride resulted in more flattened curves that reflected decreases in response rates (Figure 24A). In contrast, while the treatments of 1.0 mg/kg SKF 83959 alone and SKF 83959 with 0.06 mg/kg eticlopride similarly resulted in reduced response rates, the treatment of high dose eticlopride alone appeared to decrease responding to a greater extent than the administration of low dose eticlopride alone (Figure 24B).

One-way ANOVA on each of the FI 30-s indexes showed that the treatment conditions had significant effects on the numbers of total responses, $F(3, 24) = 6.54$, $p < 0.01$, reinforced responses, $F(3, 24) = 10.87$, $p < 0.001$, and PRP, $F(3, 24) = 7.17$, $p < 0.01$. Tukey's *post-hoc* tests found the treatment of SKF 83959 alone to significantly reduce the numbers of total responses, $p < 0.01$ (Figure 25A), and reinforced responses, $p < 0.01$ (Figure 25B), while not producing significant effects on the PRP (Figure 25C). The treatment of low dose eticlopride alone did not have significant effects on any of these indexes. In contrast, the condition of SKF 83959 pretreated with low dose eticlopride produced a near-significant decrease in the number of total responses relative to vehicle, $p = 0.061$, at the same time significant drops in the number of reinforced responses, $p < 0.01$ and increased PRP duration, $p < 0.01$, were observed. Under 0.06 mg/kg eticlopride treatments, significant drug effects were detected on total responses, $F(3, 24) = 3.62$, $p < 0.05$, reinforced responses, $F(3, 24) = 7.62$, $p < 0.001$, and PRP, $F(3, 24) = 3.02$, $p < 0.05$. *Post-hoc* tests showed that the treatment of SKF 83959 alone had a near-significant effect on decreasing the total responses, $p = 0.058$ (Figure 25D). It has significantly reduced the reinforced responses, $p < 0.01$ (Figure 25E), without affecting the duration of the PRP (Figure 25F). The administration of high dose eticlopride alone did not have significant effects on these

indexes despite a trend of a slight decrease in the total response. Alternatively, when SKF 83959 was pretreated with high dose eticlopride, the rats exhibited significant reductions in the numbers of total responses, $p < 0.05$, reinforced responses, $p < 0.05$, and increases in the duration of PRP, $p < 0.05$.

DRL 10-s performance. Figure 26A and Figure 26B display the effects of 0.02 mg/kg and 0.06 mg/kg eticlopride on the IRT of DRL 10-s performance respectively. One of the nine rats (#2) was removed from statistical analysis because it exhibited seizure-like symptoms during the progress of behavioral testing. As shown in Figure 26A, the injection of low dose eticlopride alone resulted in a curve very similar to that of the vehicle treatment. SKF 83959 alone reduced the height of the curve, while SKF 83959 pretreated with low dose eticlopride reduced the response rate on DRL 10-s to a lesser extent than SKF 83959 alone (Figure 26A). In contrast, the injections of high dose eticlopride alone and SKF 83959 alone both reduced the response rates on the IRT curve. And the administration of SKF 83959 pretreated with high dose eticlopride drastically decreased the response rate (Figure 26B).

In examination of the response-based indexes of the DRL 10-s task, one-way ANOVA revealed significant differences across low dose eticlopride treatments in the numbers of total responses, $F(3, 21) = 4.65$, $p < 0.05$, and non-reinforced responses, $F(3, 21) = 4.08$, $p < 0.05$, but not for the numbers of reinforced responses (Figure 27B) and burst responses (Figure 27D). *Post-hoc* analysis showed the treatment of SKF 83959 alone to reduce the total response, $p < 0.05$ (Figure 27A), and non-reinforced response, $p < 0.05$ (Figure 27C), on the DRL 10-s task. The respective treatments of low dose eticlopride alone and SKF 83959 pretreated with low dose eticlopride did not produce significant effects on the response-based indexes, although a trend of decrease was observed in the latter treatment. Apart from these response-based indexes, one-way

ANOVA has found significant differences across treatment conditions on the peak rate of DRL 10-s performance, $F(3, 21) = 8.10$, $p < 0.001$. As shown in Figure 28A, Tukey's *post-hoc* test indicated that the rats performed with significantly reduced peak rates when they were treated solely with SKF 83959, $p < 0.01$. The conditions of low dose eticlopride alone and SKF 83959 pretreated with low dose eticlopride did not produce significant effects on peak rate, although the latter treatment produced a trend of decrease. One-way ANOVA did not find significant differences in peak time (Figure 28B) or MRE (Figure 28C) across the treatment conditions with low dose eticlopride.

Under the treatment conditions of high dose eticlopride, one-way ANOVA yielded significant drug effects in all of the response-based indexes across conditions: total responses, $F(3, 21) = 27.27$, $p < 0.001$, reinforced responses, $F(3, 21) = 16.14$, $p < 0.001$, non-reinforced responses, $F(3, 21) = 9.91$, $p < 0.001$, and burst responses, $F(2, 14) = 5.03$, $p < 0.05$. The sole administration of SKF 83959 significantly reduced the numbers of total responses, $p < 0.001$ (Figure 27E), reinforced responses, $p < 0.05$ (Figure 27F), non-reinforced responses, $p < 0.05$ (Figure 27G), and burst responses, $p < 0.05$ (Figure 27H). The sole administration of high dose eticlopride did not have significant effects on these response-based indexes. The administration of SKF 83959 with high dose eticlopride pretreatment reduced the numbers of total responses, $p < 0.001$, and reinforced responses, $p < 0.001$, to a greater extent than the effects of SKF 83959 alone. It actually diminished the average numbers of non-reinforced response down to 1.25, $p < 0.001$, and burst responses to zero. One-way ANOVA has also found significant differences in peak rate, $F(3, 21) = 39.99$, $p < 0.001$, peak time, $F(3, 21) = 9.81$, $p < 0.001$, and MRE, $F(3, 21) = 5.22$, $p < 0.01$, across the treatment conditions. Tukey's *post-hoc* test found the sole treatment of SKF 83959 to significantly reduce the peak rate, $p < 0.01$ (Figure 28D), without significantly

affecting the peak time (Figure 28E) or the MRE (Figure 28F). The administration of high dose eticlopride alone did not produce significant effects on DRL 10-s peak rate, peak time, or the MRE. Alternatively, the treatment of SKF 83959 with high dose eticlopride significantly reduced the peak rate, $p < 0.001$, while it significantly increased the peak time, $p < 0.01$, and the MRE ratio, $p < 0.01$.

Co-administration of SCH 23390 and eticlopride pretreatment.

FI 30-s performance. Figure 29 illustrates the FI 30-s IRT curve under the combined treatments of 0.02 mg/kg SCH 23390 and 0.02 mg/kg eticlopride. The co-administration of SCH 23390 and eticlopride resulted in a scallop-shaped curve slightly below that of the vehicle treatment. While the administrations of SKF 83959 alone and SKF 83959 with the pretreatment of both antagonists resulted in flattened curves that indicated drug-induced drops in response rates.

Significant between-treatment differences were detected in the total responses, $F(3, 24) = 10.48$, $p < 0.001$, reinforced responses, $F(3, 24) = 13.23$, $p < 0.001$, and the duration of PRP, $F(3, 24) = 4.82$, $p < 0.01$. Tukey's *post-hoc* analysis revealed that under the sole administration of SKF 83959, the rats exhibited significantly reduced numbers of total responses, $p < 0.01$ (Figure 30A), and reinforced responses, $p < 0.01$ (Figure 30B); the PRP was not significantly affected (Figure 30C). The co-administration of SCH 23390 and eticlopride did not produce significant effects on these indexes of FI 30-s performance. Alternatively, under the administration of SKF 83959 with the combined DA antagonists, the rats showed significantly reduced numbers of total responses, $p < 0.001$, reinforced responses, $p < 0.001$, and increased PRP, $p < 0.01$.

DRL 10-s performance. Figure 31 shows the DRL 10-s IRT curve under the combined treatments of 0.02 mg/kg SCH 23390 and 0.02 mg/kg eticlopride. The co-administration of low

dose SCH 23390 and eticlopride did not greatly alter the typical shape of the DRL 10-s IRT. However, the treatments of SKF 83959 alone and SKF 83959 pretreated with both antagonists shifted the IRT curve in the downward direction, which indicate drug-induced decreases in response rates.

Across the drug treatments, one-way ANOVA has found significant differences in the numbers of total responses, $F(3, 21) = 25.77$, $p < 0.001$, reinforced responses, $F(3, 21) = 21.90$, $p < 0.001$, non-reinforced responses, $F(3, 21) = 17.04$, $p < 0.001$, burst responses, $F(3, 21) = 9.58$, $p < 0.001$, and peak rate, $F(3, 21) = 51.65$, $p < 0.001$. The *post-hoc* analysis found the treatments of SKF 83959 alone to produce significant reductions in total responses, $p < 0.001$ (Figure 32A), reinforced responses, $p < 0.001$ (Figure 32B), non-reinforced responses, $p < 0.001$ (Figure 32C), burst responses, $p < 0.01$ (Figure 32D), and peak rate, $p < 0.001$ (Figure 33A). The co-administration of low dose SCH 23390 and eticlopride did not produce significant effects in these response-based indexes or the peak rate. The effects of SKF 83959 pretreated with high dose both DAR antagonists on the response-based indexes and peak rate were very similar to those of the SKF 83959 treatment alone, in producing significant reductions in total responses, $p < 0.001$, reinforced responses, $p < 0.01$, non-reinforced responses, $p < 0.001$, burst responses, $p < 0.01$, and peak rate, $p < 0.001$. Finally, there were no significant differences in peak time (Figure 33B) and MRE (Figure 33C) across the treatment conditions.

Discussion

The present study yielded five main findings. First, the treatments of SKF 83959 were found to reduce the response rates on both the FI 30-s and DRL 10-s schedules of reinforcement in a dose-dependent manner. Second, SKF 83959 did not affect the index of peak time on the DRL 10-s schedule at the effective dose (1.0 mg/kg) that reduced response rates. Third, SKF 83959 did not affect the locomotor activity in terms of speed and distance, which excluded the possibility that the observed declines in response rates after drug treatments were attributed to impaired motor functions. Fourth, the examination of changes in protein phosphorylations by SKF 83959 showed a schedule-specific and brain region-specific pattern, in which the levels of pCaMKII and pCREB in the NAc were decreased in the FI 30-s group and increased in the DRL 10-s group relative to the respective control groups. The levels of pCaMKII, PKA, and pCREB were found to be positively correlated in the PFC, DS, or NAc on either or both of the scheduled-controlled tasks, whereas the levels of pERK were not correlated with these target proteins in most cases. Finally, as shown in Experiment 4, neither of the pharmacological antagonism of SKF 83959 by the pretreatments of SCH 23390 alone (0.02 and 0.06 mg/kg) nor SCH 23390 with eticlopride combined (both drugs administered at 0.02 mg/kg) appeared to successfully reverse the behavioral effects of SKF 83959 on both operant schedules. Alternatively, the pretreatment of eticlopride alone at the lower dose (0.02 mg/kg) appeared to exhibit some partial effects in reversing the behavioral effects of SKF 83959 on the total responses and peak rate. This partial reversal effect was not observed when eticlopride was given at a higher dose (0.06 mg/kg). Each of these main findings is outlined in details with further discussion below.

SKF 83959 on Operant Behavioral Performance: A Comparison with Other DA Agents

Given the present classification of dopaminergic compounds by their efficacy to stimulate AC, there have not yet been clear relationships between the types of DA agents and their behavioral effects. However, comparing the behavioral effects on operant schedules of the atypical SKF 83959 with previously tested DA agents may provide novel information regarding its functions *in vivo*, especially when very few studies have examined its effects on FI or DRL schedule-controlled behaviors.

Response rate. According to the principle of response rate dependency in behavioral pharmacology, the effects magnitude of a particular drug on response rates would be dependent on the baseline rate in the absence of that particular drug (Dews, 1955; Branch, 1984). McMillan (1969) tested the effects of *d*-amphetamine on FR and FI schedules of reinforcement; it was found that *d*-amphetamine at low doses increased the responding on schedules that generated low rates (FR 250 and FI 5-min), and decreased the respective responding at high doses. On schedules that produced high response rates (FR 30 and FI 60-s), *d*-amphetamine did not affect responding at low doses but reduced the respective responding at high doses (McMillan, 1969). Hence it was suggested that drug effects were more related to the baseline rates of responding rather than the types of reinforcement schedules (McMillan, 1969). In the present study, the FI 30-s schedule generated higher response rates than the DRL 10-s schedule, hence the respective schedule-controlled behaviors were expected to be differentially affected by drug treatments.

Early studies have reported the administration of amphetamine to increase operant responding at low doses, and reduce responding at higher doses (De Oliveira & Graeff, 1972; Barrett, Miller, Dohrmann, & Caine, 2004). On FI schedules, amphetamine-related compounds have been reported to induce a characteristic pattern of initially increased response rates during

the intervals (Maricq, Roberts, & Church, 1981). Alternatively, the administration of 1.0 mg/kg amphetamine was also found to increase the total responses on the DRL 10-s schedule (Cheng & Liao, 2007). Neither of the amphetamine-induced effects resembled the present findings with SKF 83959 treatments. On the other hand, contrary to the effects of amphetamine, the administration of selective DAR agonists and antagonists has generally been found to reduce the overall response rates (Rusk & Cooper, 1988, 1989, 1994; Drew et al., 2003; Barrett et al., 2004). Barrett et al. (2004) tested the effects of SKF 82958 and R-6-Br-APB (D_1R agonists), 7-OH-DPAT and quinelorane (D_2R agonists), SCH 39166 (D_1R antagonist), and eticlopride (D_2R antagonist) on operant responding for liquid food as reinforce in rats. All of the DA agents tested were found to reduce responding in a fashion dependent on the drug dose and reinforcer magnitude. In general, both D_1R- and D_2R-selective agents similarly reduced the response rate, which does not comply with the general assumption that receptor agonists and antagonists exert opposing effects on behavioral performance. Hence the cellular mechanisms that underlie pharmacological manipulations are not as straightforward as first anticipated. Moreover, the performance of schedule-controlled behaviors in the above studies likely involved both classes of DARs, as the enhanced or disrupted DA transmission of either class of receptors yielded a common reduction in response rates.

In the present study, the administration of 1.0 mg/kg SKF 83959 reduced the total responses on the FI 30-s schedule by approximately 6 folds, and decreased that on the DRL 10-s schedule by about 2 folds. The observed difference in drug effects may be attributed to possible task-dependent differences between the two operant schedules. From experiments on monkeys, Platt et al. (2000) reported that SKF 83959 injections dose-dependently reduced the operant response rates on a FI 3-min shock termination task; the administration of 0.3 mg/kg SKF 83959

reduced the response rates by about 2 folds relative to control, whereas that of 3.0 mg/kg SKF 83959 almost completely diminished the response rates. These reported data are consistent with the present results, which also observed reductions in response rates on the FI 30-s schedule in appetitive conditioning. Similar to its effects on FI schedules, SKF 83959 also decreased the response rates and peak rates in a dose-dependent manner on the DRL 10-s schedule in the present study. The effects of SKF 83959 on DRL schedule-controlled behaviors have not been tested in previous research.

It was also noted in Experiment 1, a greater proportion of the rats in the FI 30-s group ceased to respond upon 1.0 mg/kg SKF 83959 injections (3 of 12 rats) relative to that in the DRL 10-s group (1 of 11 rats). This may also suggest possible task-dependent differences in the levels of sensitivity to the drug.

Locomotor activity. As reported by early studies, compounds that increase brain DA transmission, such as the indirect DA agonists amphetamine and cocaine, and the non-selective DA agonist apomorphine, have been observed to increase the locomotor activity in animals (Smith, 1965; Cole, 1978; Castro, Abreu, Calzadilla, & Rodriguez, 1985). Also, the administrations of the D_1R full agonists (SKF 82958 and SKF 81297), and the D_1R partial agonists (SKF 75670, SKF 77434, and SKF 38393) have been found to increase the locomotor activity (Meyer & Shults, 1993; Halberda, Middaugh, Gard, & Jackson, 1997; Schindler & Carmona, 2002; Desai, Terry, & Katz, 2005). It is noted that the aforementioned effects appeared to be at smaller magnitudes than that of cocaine, and the drug-induced changes in activity levels over drug doses or time do not necessarily follow similar patterns. Moreover, mixed results regarding the effects of D_2R agonists on locomotor activity have been reported. Some studies have reported quinpirole to reduce the locomotor activity (Mattingly, Rowlett, & Lovell, 1993;

Halberda et al., 1997), while others have reported quinpirole and bromocriptine to produce biphasic effects on locomotor activity (Eilam & Szechtman, 1989; Hoffman & Wise, 1992). Regarding DAR antagonists, the locomotor activity was consistently reduced by both of D_1R antagonists, (SCH 23390 and SCH 39166; Hoffman & Beninger, 1985; Schindler & Carmona, 2002; Collins et al., 2010) and D_2R antagonists (raclopride and eticlopride; Hillegaart & Ahlenius, 1987; Schindler & Carmona, 2002; Collins et al., 2010).

In a pharmaco-ethological study by Deveney and Waddington (1995), rats injected with SKF 83959 did not exhibit any drug-induced changes on locomotor activity across the tested dose range (0.01 to 1.25 mg/kg), despite some increases in rearing at the higher tested doses. The microinjection of SKF 83959 into the NAc by Cools et al. (2002) did not have any effects on locomotor activity. Furthermore, the local infusion of SKF 83959 into the NAc counteracted the increase in locomotor activity induced by SKF 81297, which indicated that SKF 83959 resembled the D_1R antagonist SCH 39166 (Cools et al., 2002). Similarly, Peacock and Gerlach (2001) did not find SKF 83959 to induce any motor unrest in monkeys. In contrast, Perreault et al. (2010) reported that chronic SKF 83959 administrations (0.4 mg/kg; 7 days) increased locomotor activity. This SKF 83959-induced increase in locomotor activity was significant but to a less extent than that of the D_1R agonist SKF 83822, and it was only observed on days 2 and 3 of the seven days (Perreault et al., 2010).

Overall, the present experiment did not find the injections of SKF 83959 at 0.5 or 1.0 mg/kg to alter the locomotor activity in terms of travelled speed and distance relative to vehicle control. The present finding is consistent with the results from Deveney and Waddington (1995) and Cools et al. (2002). Thus, the primary concern that 1.0 mg/kg SKF 83959 produced motor deficits that affected operant responding was eliminated.

Reinforcement Motivation. The index of PRP on FI schedules refers to the duration of time lapse from receiving a reinforcer to making the next lever press in the following interval. The PRP has been reported to increase with increased durations of FI schedules (Chung & Neuringer, 1967; Shull, 1970). Similarly, on FR schedules, the length of PRP has been reported to increase with decreased response rates and increased ratio requirements (Ferster & Skinner, 1957; Felton & Lyon, 1966). In the behavioral tests of Experiment 1 and Experiment 2, the sole treatment of SKF 83959 prolonged the PRP on the FI 30-s schedule, which may suggest that the drug reduced the subject's intrinsic interest in the reinforcer. The numbers of reinforced responses in SKF 83959-treated rats on the FI 30-s schedule were consistently reduced to between 1/3 and 2/3 of that under vehicle treatment, which may be a plausible indication of reduced interest or drive to obtain the reinforcer. In addition to the possible effects on motivation, SKF 83959 may have also interfered with the sense of reinforcement durations, since the PRP is characteristic of the inter-reinforcement durations on FI schedules.

Timing performance. The pharmacological manipulation of brain DA transmission is known to modulate temporal regulation. Previously, the D_1R agonist SKF 38393 and D_2R agonist quinpirole were found to reduce the peak time on peak interval procedures of 40-s (Frederick & Allen, 1996). Alternatively, the respective administrations of D_1R antagonists (SCH 23390 and SKF 83566), and D_2R antagonists (eticlopride and haloperidol) at low doses did not affect the timing performance on peak interval procedures (Frederick & Allen, 1996; Drew et al., 2003; Cheung et al., 2006). The administration of haloperidol at higher doses induced the performance of overestimated intervals on the peak interval procedure with 12-s and 36-s (Drew et al., 2003). Based on these findings, it was generally hypothesized that the effects of DA agonists on timing were similar to those of stimulant compounds like amphetamine, as they

tended to speed up the internal clock and shifted the IRT curve to the left. In contrast, DA antagonists tended to slow down the internal clock and shifted the IRT curve to the right, at a potency that was predictable by their affinity of binding to D_2Rs (Maricq & Church, 1983; Meck, 1986). In examination of the FI 30-s IRT curves from the present study, SKF 83959 did not appear to produce horizontal shifts in the curves, which resulted in a distinct IRT pattern from those of the DA agents tested in previous studies.

The peak time measure on the DRL schedule may provide further details in regard to the relationship between SKF 83959 and timing performance. In the present study, SKF 83959 alone did not affect the peak time on the DRL 10-s schedule at all tested doses throughout the experiments. The present lack of SKF 83959-induced alterations on DRL 10-s peak time demonstrated the unique effects of this novel compound on timing performance, in comparison to the previously tested stimulant drugs and D_1R agonists that shifted the IRT curve leftward (Cheng & Liao, 2007; Frederick & Allen, 1996), or the D_2R antagonists that shifted the IRT curve rightward (Drew et al., 2003). The mechanism that distinguishes the difference between SKF 83959 and other DAR agonists is unknown at this point. However, it may be related to the unique pharmacological properties from SKF 83959-coupled signaling pathways.

Summary. The present behavioral results showed the injections of 1.0 mg/kg SKF 83959 to effectively reduce the operant response rates and increase the FI 30-s PRP in Experiment 1 and Experiment 2. This dose treatment did not affect the locomotor activity or the DRL 10-s peak time. Overall, SKF 83959 appeared to exert more suppressive effects on the behaviors tested in the present study than stimulatory effects, which did not resemble those of typical DAR agonists.

The Involvement of Brain Regions in the Operant Behavioral Changes Induced by SKF 83959

An inspection of the previous studies that have manipulated different brain regions and tested their effects on operant performance may provide useful insights into the behavioral effects of SKF 83959 in the present study.

Prefrontal cortex. From studying the behavioral deficits induced by PFC lesions, this brain region is known to associate to the higher cognitive functions including selective attention, working memory, planning, and executive control (Wall & Messier, 2001; Curtis & D'Esposito, 2003; Baker et al., 1996). The PFC has also been hypothesized to exert behavioral inhibition on tasks including the DRL schedule-controlled behaviors. Sokolowski and Salamone (1994) reported that rats depleted of DA transmission in the mPFC by 6-OHDA treatments exhibited increased responding during IRTs of short durations along with reduced efficiency at obtaining reinforcers on the DRL 30-s schedule of reinforcement. This reported finding supported the notion that disrupted mPFC functioning interfered with the behavioral inhibition over frequent responding, thus resulted in increased responses.

Pre-training lesions in the mPFC rendered rats insensitive to devalued rewards, whereas post-training lesions did not have this effect (Ostlund & Balleine, 2005). These data suggest that the mPFC is involved in the acquisition rather than the expression of response-outcome associations. Since the present pharmacological manipulations were conducted in the post-training phase, presumable drug-induced alterations in PFC functioning would not be necessarily reflected on the expression of observable behaviors.

Dorsal striatum. The striatum is the primary input of neural signals coming from the cerebral cortex into the basal ganglia. It is known to possess pivotal functions in motor control. Moreover, recent cognitive models have proposed the formation of corticostriatal networks by neural connections between the PFC, sensorimotor cortex, and DS that implicate in the functions of decision-making, goal-directed learning, reward-mediated processes, and habitual learning (Balleine, Delgado, & Hikosaka, 2007; Wickens, Budd, Hyland, & Arbuthnott, 2007). Within the DS, the dorsomedial striatum was hypothesized to receive projections from the prelimbic mPFC and regulate goal-directed learning. Lesions in the dorsomedial striatum disrupted action-outcome learning and rendered rats insensitive to devalued rewards (Yin, Ostlund, Knowlton, & Balleine, 2005). Conversely, the dorsolateral striatum was hypothesized to work with the infralimbic mPFC and sensorimotor cortex in processing habitual learning, as lesions in this area reversed the insensitivity to devalued rewards in overtrained rats (Yin, Knowlton, & Balleine, 2004). Notice that these reports on the functions of the dorsomedial and dorsolateral striatum emphasized their significance in the acquisition and habit formation of instrumental behaviors. As described above for the mPFC, the present experiments only examined the effects of SKF 83959 on post-training behavioral performance instead of acquisition. Hence the involvement of the DS may not have been the most critical in the performance of schedule-controlled behaviors. Furthermore, microinjections of the DA antagonist EEDQ into the DS did not produce any changes in response rates on FI schedules of reinforcement (Cory-Slechta, Pazmino, & Bare, 1997), suggesting that impaired DS functions did not provide the primary explanation for the presently observed reduction in response rates on the FI 30-s schedule by SKF 83959.

The corticostriatal networks also possess important functions in interval timing; lesions in the DS impaired the abilities of temporal control on time-related tasks such as the peak interval

procedure (Gibbon, Malapani, Dale, & Gallistel, 1997; Meck, 2006; Meck, Penney, & Pouthas, 2008). Since the peak time was not significantly affected by SKF 83959 in the present experiments, the current data imply that the DS was not the primary target of SKF 83959 actions.

Nucleus accumbens. Ever since the proposal of the anhedonia hypothesis (Wise, 1982), findings from studies that interfered with DA transmission in the NAc have considered this brain region as a critical part of the reward circuitry that mediated the reinforcing effects of rewarding stimuli like food (Hernandez & Hoebel, 1988) and substances of abuse (Caine & Koob, 1994). However, more recent evidence have reported DA depletions in the NAc to exert less or no effects on the reinforcement schedules that generated lower baseline rates or had smaller work requirements (Cousins, Trevitt, Atherton, & Salamone, 1999; Aberman & Salamone, 1999). Such findings suggested that the NAc may be implicated in the effort- or work-related response allocation rather than the positive reinforcing effects as previously interpreted (Salamone, Cousins, & Snyder, 1997). Schultz et al. (1997) discussed the involvement of the mesolimbic DA system in reward prediction, with a main focus on the VTA-NAc pathway. In monkeys, single-cell recordings on the VTA DA neurons, which presumably increase the DA release in the NAc, have detected short and phasic firing patterns when monkeys were in contact with rewarding stimuli. And most importantly, the firing could be shifted from the time of reward delivery to that of cue presentation when the reward was repeatedly paired with originally neutral cues (Schultz et al., 1997). Hence any deficits in normal VTA-NAc functions that impair the ability to predict incoming rewards may also alter the response patterns on a reinforcement schedule.

The local infusion of 6-OHDA into the NAc reduced rat's responding on the FI 60-s schedule of reinforcement (Robbins, Roberts, & Koob, 1983), which suggested that DA neurotransmission in the NAc was critically involved in the performance of FI schedule-

controlled behaviors. Similarly, Cory-Slechta et al. (1997) found that response rates on the FI 120-s schedule was reduced by the microinjection of the DA antagonist EEDQ into the NAc. Such effects were restored with the respective pretreatment of SCH 23390 or eticlopride, suggesting that interactions between NAc D_1Rs and D_2Rs may be implicated in the operant performance under FI schedules. In the present study, peripheral injections of SKF 83959 reduced the response rate on the FI 30-s schedule, which resembled the rate-reducing behavioral effects of DA-depleted NAc. Such an inference can also be applied to the present data from the DRL 10-s schedule, as SKF 83959 also reduced the DRL 10-s response rates and peak rates.

In respective to the functions related to temporal processing, Meck (2006) reported that lesions in the NAc did not affect the accuracy of temporal performance on the peak interval procedures of 10-s and 60-s, while the discrimination of reward values was impaired. Overall, these previous reports on the response rate and peak time performed by rats with NAc lesions are consistent with the present interpretation that SKF 83959 likely affected the functions of the NAc.

Dorsal hippocampus. The hippocampus has been well-explored for its functions in memory consolidation of spatial and/or temporal learning. Moreover, Meck and Church (1984) investigated the effects of bilateral lesions of the fimbria-fornix, which consisted of nerve fibres connected to the hippocampus, on working memory during temporal differentiation by training rats to respond on differential levers upon the presentation of specific duration- or rate-based auditory signals for reinforcers (eg. press on the left lever upon a short signal and the right lever for a long signal). The rats went on to receive signals of intermediate durations or rates, for which their pre-lesion and post-lesion choices of operant responding were compared (Meck & Church, 1984). The rats with lesioned fimbria-fornix exhibited a leftward shift in their proportion of right lever presses as a function of signal durations or rates, which suggested an impaired

memory on the value of reinforced durations or rates (Meck & Church, 1984). By training rats on FI 50-s schedules and using the peak interval procedure, Olton, Meck, and Church (1987) found the peak time performance of rats with lesions in the fimbria-fornix to be significantly shorter than that before lesion. In contrast, the peak rate before and after lesion operations were not significantly different (Olton et al., 1987). Hence the rate-reducing effects of SKF 83959 on the FI 30-s schedule in the present study did not resemble the effects of hippocampal lesions as reported by Olton et al. (1987). In the examination of DRL-controlled behaviors, Clark and Isaacson (1965) reported that hippocampal lesions increased the response rates on the DRL schedule. This pattern was also different from the observation of reduced peak rates by SKF 83959 in the present study.

Summary. The present results suggested the effects of SKF 83959 on operant behaviors to resemble those of lesions in the NAc, rather than lesions in the PFC, DS, or hippocampus. Recent research suggested that impaired DA transmission in the NAc would disrupt the willingness of the animals to fulfill the work effort required for obtaining reinforcers, without compromising the appetitive motivation. For example, Nowend, Arizzi, Carlson, and Salamone (2001) reported that local infusions of D_1R or D_2R antagonists to the NAc reduced lever-pressing for food. However, the decrease in the amount of obtained food from the operant task was compensated by the increased intake of freely available chow (Nowend et al., 2001). Together, it was thus hypothesized that the present SKF 83959 treatment most likely interfere with DA transmission in the NAc, which affected the rats' motivation to lever-press for reinforcers rather than their abilities to process time-based or other cognitive-like stimuli.

The Involvement of Signaling Proteins in the Operant Behavioral Changes Induced by SKF 83959

As shown by the present protein results, SKF 83959 induced prominent changes in the levels of pCaMKII, PKA, and pCREB in the NAc on both operant schedules. The levels of the phosphorylated proteins were reduced by SKF 83959 in the FI 30-s group but enhanced in the DRL 10-s group. The levels of pCaMKII and pCREB were positively correlated with each other in the DS and NAc , but not in the PFC or hippocampus. The levels of PKA and pCaMKII were positively correlated with each other in the PFC on both schedules. In contrast, the phosphorylation of ERK was not particularly affected by SKF 83959 treatments in both groups, nor was it significantly correlated with the other proteins tested.

CaMKII. CaMKII is an abundant protein inside neurons whose activation is regulated by calcium ions. Studies on CaMKII have originally focused on its functions in linking calcium signals to synaptic plasticity and long-term potentiation (LTP) or long-term depression (LTD) in the hippocampus during the NMDAR signaling cascade (Fink & Meyer, 2002). Wiltgen, Law, Ostlund, Mayford, and Balleine (2007) investigated the effects of striatal CaMKII activation by using transgenic mice that constitutively expressed active CaMKII in the striatum, and testing their performance on tasks of classical as well as instrumental conditioning. The transgenic mice did not perform differently from wildtype mice on the acquisition of reward-paired Pavlovian cues or operant behaviors, nor were there any differences between mice in the responses to devalued rewards or degraded schedule contingency (Wiltgen et al., 2007). However, the active expression of striatal CaMKII impaired the process of Pavlovian-instrumental transfer, such that the transgenic mice showed deficits in operant responding upon the presentation of Pavlovian cues, suggesting that CaMKII is critical in forming the associations between the Pavlovian cues

with the instrumental responses to obtain reinforcers (Wiltgen et al., 2007). These findings support the notion that CaMKII activation is involved in the learning of reward motivation-mediated behaviors in addition to the modulation of synaptic plasticity.

Perreault et al. (2010) tested the effects of repeated SKF 83959 injections (0.4 mg/kg) on CaMKII expression in the NAc at 60 minutes after drug injections. Their data showed that SKF 83959 did not affect pCaMKII levels but significantly reduced the levels of native CaMKII, suggesting that low doses of chronic SKF 83959 injections affected the levels of native CaMKII rather than the phosphorylation. Using immunohistochemistry, Rashid et al. (2007) reported that the acute injections of SKF 83959 (3 mg/kg) or quinpirole (2 mg/kg) alone did not increase the proportion of pCaMKII-labelled cells in rat NAc within 20 minutes after drug treatments; it took the co-administration of SKF 83959 and quinpirole to induce a rapidly marked increase in NAc pCaMKII levels. Conversely, Ng, Rashid, So, O'Dowd, and George (2010) found SKF 83959 treatments (1.0 mg/kg) *in vivo* to significantly increase pCaMKII levels in mice striatum at 30, 60, and 90 minutes after drug injections. Hence it was inferred that the acute injections of SKF 83959 at moderate doses (1.0 mg/kg) could activate pCaMKII in the NAc after at least 30 minutes of drug action, and the activation of pCaMKII by SKF 83959 could be potentiated with the co-administration of quinpirole.

In the present study, the SKF 83959 treatment decreased the levels of NAc pCaMKII on the FI 30-s schedule, whereas it increased that on the DRL 10-s schedule. This contradictory observation was attributed to possible differences from the behaviors maintained on the distinctive schedules. As the FI 30-s schedule induced a relatively higher rate of responding than the DRL 10-s, the different criteria for motivation and work efforts may have led the brain to respond differently to the same drug treatments, as a result of neural adaptations to external

stimuli or behavioral history (Olsen, 2011). On the other hand, the present study has found positive correlations between the levels of pCaMKII and pCREB within the DS and NAc on both schedules of reinforcement. This observation suggests that there may be interactions between pCaMKII and pCREB signaling in the mechanism underlying operant performance.

ERK. ERK expression in the striatum is implicated in processes of learning and decision-making, especially in the contexts of reward-related cues, that makes it a topic of much research on problems related to drug addiction (Shiflett & Balleine, 2011). The acute administration of cocaine was found to increase the phosphorylation of ERK in the striatum via a mechanism coupled to the combined activation of both D_1R and NMDAR (Valjent et al., 2000). The inhibition of ERK activation by the selective ERK inhibitor SL327 was found to decrease the cocaine-induced hyperlocomotion and place conditioning (Valjent et al., 2000). Moreover, the inhibition of ERK impaired the responding to a conditioned stimulus on a Pavlovian-instrumental transfer task, in which the rats were trained to respond for food upon an auditory cue (Shiflett et al., 2008). As it appears, ERK signaling in the striatum is a mediator of functions related to motivation, learning, and memory, by which the D_1R and NMDAR exert regulatory control over neuroplasticity.

In the present study, the levels of total pERK in the brain regions tested were not significantly altered by SKF 83959. This observation corresponds to the initial hypothesis that SKF 83959 acts on D_1-D_2 receptor heteromers rather than D_1Rs and NMDARs, thus the present data further support the claim that SKF 83959 does not co-activate the D_1R and NMDAR *in vivo*.

PKA. PKA is a cAMP-dependent protein coupled to the activation of typical D_1Rs in the NAc, whose function is also related to reward-mediated learning (Beninger & Gerdjikov, 2004). The bilateral microinjection of the PKA inhibitor (Rp-cAMPs) or activator (Sp-cAMPS) into the

NAc impaired the acquisition of operant responding for food on ratio-based schedules of reinforcement, which indicated the necessity of NAc PKA in optimal levels for appetitive learning (Baldwin, Sadeghian, Holahan, & Kelley, 2002). In addition to its critical role in learning, PKA was also implicated in the expression of drug self-administration. The activation of PKA in the NAc increased the operant responding for cocaine on both FR and progressive ratio (PR) schedules of reinforcement, while the inhibition of NAc PKA reduced the responding for cocaine on PR schedules (Self et al., 1998; Lynch & Taylor, 2005). It was thus suggested that PKA activity in the NAc was related to the motivation for obtaining cocaine and possibly other addictive substances (Lynch & Taylor, 2005).

The present study only measured the levels of PKA rather than its activation. The levels of PKA were negatively correlated with SKF 83959 administration in the PFC and NAc on the DRL 10-s schedule and FI 30-s schedules respectively. And the levels of PKA were positively correlated with those of pCaMKII or pCREB in the PFC, DS, and NAc. Despite the claims from previous *in vitro* reports that SKF 83959 does not activate the typical AC-coupled D_1Rs (Andringa, Drukarch, Leysen, Cools, & Stoof, 1999; Rashid et al., 2007), the present patterns of correlation from *in vivo* experiments cannot rule out the potential involvement of PKA in the behavioral effects of SKF 83959. The drug-induced alterations in PKA levels may be attributed to possible intracellular cross-talks between downstream signaling proteins. Future assays of the PKA activity with commercial kits (Gigante, Santerre, Carter, & Werner, 2014) or by the phosphorylation of its downstream proteins such as DARPP-32 (Lynch, Kiraly, Caldarone, Picciotto, & Taylor, 2007) may provide a more direct examination of the effects of SKF 83959 on PKA activation.

CREB. CREB is a transcription factor that regulates the neuroplasticity underlying learning and memory, whose activity can be affected by a complex array of neural signals from pathways involving CaMKs, cAMP-PKA, and MAPK/ERK (Carlezon, Duman, & Nestler, 2005; Nishi et al., 2008). Calcium signals appeared to exert differential control over CREB activity; CaMKIV has been reported to phosphorylate CREB at serine-133, which induces CREB dimerization that goes on to activate CREB and the subsequent gene transcription (Dash, Karl, Colicos, Prywes, & Kandel, 1991; Carlezon et al., 2005). Alternatively, CaMKII has been reported to phosphorylate CREB at serine-133 and serine-142, the latter of which induces the dissociation of CREB dimers and decreases the CREB-induced gene transcription (Sun, Enslen, Myung, & Maurer, 1994; Wu & McMurray, 2001). On the other hand, the cAMP-PKA and MAPK/ERK signaling have also been reported to activate CREB via phosphorylation at serine-133 (Gonzalez & Montminy, 1989; Dash et al., 1991; Xing, Ginty, & Greenberg, 1996). The present results from Experiment 2 showed the levels of pCREB to positively correlate with that of pCaMKII and PKA in the DS and NAc, which suggested that the effects of SKF 83959 on operant behaviors may be related to the alterations in these signaling proteins. While previous *in vitro* experiments have found CaMKII to modulate CREB activity in differential ways via phosphorylation at different sites, the present study has observed a positive correlation between the levels of pCaMKII and pCREB in the DS and NAc of the rats tested *in vivo*.

In addition to learning and memory, CREB is also implicated in drug addiction. The levels of CREB activity were found to be increased by chronic exposure to addictive drugs like cocaine (Mattson et al., 2005). Using techniques of virus-mediated gene overexpression, Larson et al. (2011) reported that increased CREB expression in the NAc enhanced the operant responding for cocaine on both FR and PR schedules of reinforcement, whereas the decrease in

NAc CREB expression had the opposite effects. Thus it was inferred that altered levels of CREB activity in the NAc could impair the performance of reward-motivated behaviors, as also observed in the present study.

Summary. The changes in protein levels induced by SKF 83959 were mainly found in the DS and NAc. The observation of relatively little protein changes in the PFC and hippocampus suggest that these brain regions were unlikely to be the primary targets of SKF 83959. Although both of CaMKII and ERK expression in the NAc are implicated in cellular excitability and the formation of associations between Pavlovian cues and instrumental responses, the present study found SKF 83959 to affect the phosphorylation of CaMKII more so than that of ERK. This observation supported the initial hypothesis that the stimulation of D_1-D_2 heteromers by SKF 83959 would activate CaMKII via the D_1-D_2 receptor heteromer-coupled signaling cascade of PLC, PI hydrolysis, and calcium ions, rather than the NMDAR-coupled ERK signaling pathway. From present observations, the SKF 83959-induced protein phosphorylations (pCaMKII and pCREB) appeared to localize in the NAc and DS, which coincided with the brain regions that contain higher proportions of co-localized D_1Rs and D_2Rs in the brain (Lee et al., 2004; Ng et al., 2010). SKF 83959 was observed to induce changes in the levels of native proteins in some of the brain regions tested, which were expected to be affected by chronic manipulations rather than acute drug injections. It was noted that in the FI 30-s group, the three rats with no responses after high dose SKF 83959 treatment in Experiment 1 were placed into the vehicle group in Experiment 2, while the other two rats with less than 2 lever presses in Experiment 1 were placed into the experimental group in Experiment 2. For the DRL 10-s group, the one rat with no responses after high dose SKF 83959 treatment was also placed into the vehicle group in Experiment 2. It was speculated that the differences in native protein levels may

be related to the levels of sensitivity to high dose SKF 83959 in the first exposure to the compound, or to possible interactive effects between the second challenge with drug injections and the behavioral training.

DAR Antagonists and the Operant Behavioral Changes Induced by SKF 83959

Experiments of pharmacological antagonism can be applied to investigate the cellular mechanisms that underlie particular behavioral functions via the blockade of selective receptors or neurotransmission. Substantial studies that applied D_1R- and D_2R-selective antagonists alone or with other DA-related drugs have contributed greatly to the understanding of the differential functions of D_1Rs and D_2Rs.

Previous research. The effects of amphetamine on stimulating locomotor activity and inducing behavioral sensitization could be blocked by the administrations of either D_1R- or D_2R-selective antagonists (Vezina, 1996; Chausmer & Ettenberg, 1999; Cheng & Liao, 2007). For example, the amphetamine-induced increase in responding and shortened peak time on the DRL 10-s schedule were no longer observed when SCH 23390 or raclopride was co-administered; it was thus hypothesized that the effects of amphetamine were mediated via both classes of DARs (Cheng & Liao, 2007).

Also using the strategy of pharmacological antagonism, Deveney and Waddington (1995) tested the respective pretreatments of the D_1R antagonists (SCH 23390 and BW 737C), and the D_2R antagonist (YM 09151-2) before SKF 83959 injections on the behaviors of intense grooming and vacuous chewing in rats as these were previously affected by the drug. Interestingly, the respective administrations of the D_1R and D_2R antagonists successfully reversed the SKF 83959-induced increases in intense grooming, whereas the D_1R antagonists alone failed to exhibit the same reversal effects on SKF 83959-induced increases in vacuous

chewing, and the D_2R antagonist alone actually enhanced such an effect (Deveney & Waddington, 1995). While both behaviors of intense grooming and vacuous chewing were affected by SKF 83959, the sole treatments of D_1R- or D_2R-antagonists yielded different patterns of reversal effects on these indexes. Hence the relationship between different behaviors or tasks under drug treatment and the underlying neural interactions appeared to be more complex than previously anticipated, as also observed in the present case of SKF 83959.

Focusing on biochemical changes, Rashid et al. (2007) reported that the respective pretreatments of SCH 23390 and raclopride blocked the activation of CaMKII by co-administered SKF 83959 and quinpirole *in vivo*. Moreover, D_1R -/- and D_2R -/- knock-out mice did not exhibit the same pattern of increases in CaMKII levels upon SKF 83959 and quinpirole co-administration (Rashid et al., 2007). Similarly, *in vitro* assays of the incorporation of G proteins using radioactively labelled GTP in D_1R and/or D_2R expressing HEK cells and membrane preparations from mice striata have found the co-administration of SKF 83959 with quinpirole to activate $G_{q/11}$ proteins, which was also blocked by the pretreatments of either SCH 23390 or raclopride (Rashid et al., 2007). The activation of striatal pCaMKII by SKF 83959 injections (1.0 mg/kg) *in vivo* was blocked either pretreatments of SCH 23390 (1.0 mg/kg) or raclopride (0.5 mg/kg) (Ng et al., 2010). These findings indicate the crucial involvement of both D_1Rs and D_2Rs in the pharmacological mechanism of SKF 83959. However, this observation has not been tested in behavioral models at the level of instrumental conditioning.

Present findings. In opposition to the initial hypothesis, neither doses of SCH 23390 pretreatments (0.02 or 0.06 mg/kg) had successfully reversed the SKF 83959-induced reductions in response rates on the FI 30-s and DRL 10-s schedules of reinforcement. While Ng et al. (2010) observed the respective pretreatments of SCH 23390 and raclopride to biochemically reverse the

drug-induced increases in striatal pCaMKII, it was noted that the DAR antagonists were employed in much higher doses (1.0 mg/kg and 0.5 mg/kg) relative to those in the present study (0.02 mg/kg and 0.06 mg/kg). The application of DAR antagonists at such high doses was effective in reversing the biochemical effects of SKF 83959, but it is supposed to impair the rat's behavioral performance which renders it unsuitable for use in the present study. Other studies have reported finding the pharmacological antagonism of SKF 83959-induced behaviors to show mixed patterns. For example, the induction of intense grooming by 0.05 mg/kg SKF 83959 in rats was successfully reversed by the SCH 23390 pretreatments, but such a reversal effect was not observed in vacuous chewing (Deveney & Waddington, 1995). Hence the present absence of reversal effects by SCH 23390 may be related to the factors of dose-related or task-specific differences between studies.

The present pretreatment of low dose eticlopride appeared to partially antagonize the SKF 83959-induced declines in response rates and DRL 10-s peak rates. Similar reversal effects were not observed with the pretreatment of high-dose eticlopride. Previous reports on the effects of D_2R antagonists in counteracting SKF 83959 also suggested behavior-dependent differences. For example, the pretreatments of YM 09151-2 reversed SKF 83959-induced increases in intense grooming, but it actually enhanced the increases in vacuous chewing by SKF 83959 treatments (Deveney & Waddington, 1995).

Assays of competition binding between SKF 83959 and radioactively labelled raclopride on D_2Rs in D_1-D_2 HEK cells revealed that SKF 83959 bound to D_1-D_2 HEK cells with a higher affinity than D_2 HEK cells, and this binding was not eliminated upon treatments with pertussis toxin (Rashid et al., 2007). Since Rashid et al. (2007) also reported treatments of pertussis toxin to disrupt the D_2R-mediated $G_{i/o}$ protein activation, these findings suggested that SKF 83959

bound with higher affinities to a pertussis toxin-resistant binding site on the D_2R that did not couple to $G_{i/o}$ activation. It is hypothesized that the pretreatment of 0.02 mg/kg eticlopride may have interfered with the availability of this D_2R binding site for SKF 83959, thus disrupted its antagonist-like effects on operant behaviors by exhibiting a partial reversal effect in the drug-reduced response rates. Similar effects of antagonism were not observed in the pretreatments of eticlopride at 0.06 mg/kg. Moreover, the co-administration of SKF 83959 with the high dose eticlopride greatly diminished DRL 10-s responding and significantly increased the peak time, suggesting that the dose might have been too high for the present behavioral measures.

In applying the co-administration of SCH 23390 and eticlopride, this pretreatment did not successfully restore the SKF 83959-induced declines in response rates on the FI 30-s and DRL 10-s schedules of reinforcement. This finding is not consistent with the initial hypothesis based on *in vitro* studies that SKF 83959 exerts its effects via D_1-D_2 receptor heteromer activation. It may be that the drug doses were not high enough to effectively reverse the SKF 83959-induced effects. The combinations of some more doses of SCH 23390 and eticlopride in the co-administration experiment (eg. 0.04 mg/kg SCH 23390 and 0.04 mg/kg eticlopride, 0.04 mg/kg SCH 23390 and 0.02 mg/kg eticlopride, 0.02 mg/kg SCH 23390 and 0.04 mg/kg eticlopride etc.) may need to be tested before a conclusion can be made. Alternatively, the receptor binding specificity of SKF 83959 for the D_1-D_2 receptor heteromers *in vivo* may not be as selective as previously expected from *in vitro* reports, according to the study by Chun et al. (2013).

Study Limitations

The primary limitation of the present study was the repeated administrations of combined DA agents to rats in a within-subjects design. The possible effects of drug tolerance were

minimized by adopting the Latin square design in Experiment 4 and allowing two days of retrain between drug sessions, however, the potential effects of repeated drug administrations and prolonged training periods cannot be completely ruled out. Another limitation of the present study was the lack of control groups that received either of only behavioral training or only SKF 83959 injections. Since all of the rats in the current study underwent the operant training and received the drug injections, the observed protein changes might be the combined outcome of both factors rather than any specific one. It could only be inferred that the degrees of influence from behavioral training were very similar in all of the rats, such that any differences in protein levels between the vehicle-treated and drug-treated rats were attributed to the drug effects. Additionally, the task of finding potentially effective doses of DAR antagonists that could antagonize the behavioral effects of SKF 83959 without affecting operant behaviors in Experiment 4 was challenging. The present doses of DAR antagonists used in Experiment 4 may have been too low to achieve a reversal effect; more combinations of higher doses may need to be tested. Finally, the design of the present study cannot rule out the possibility that SKF 83959 acted on receptors other than the D_1-D_2 receptor heteromers or that it interfered with DA release in other brain regions. In defense to these claims, it is argued that the dose of SKF 83959 used in the present study (1 mg/kg) was unlikely to be high enough to activate non-selective binding to other receptors. No such non-selective binding was reported in the study by Rashid et al. (2007), which tested the effects of 3 mg/kg SKF 83959 on the levels of pCaMKII expression *in vivo*. And currently, no studies have examined the DA levels *in vivo* after SKF 83959 treatments; it may also be a direction for future studies.

Suggestions for Future Studies

As the present drug administrations were only given via the peripheral route that were assumed to affect the whole brain, future work with an approach to conduct brain-region specific microinjection may provide more direct evidence on the specific sites of SKF 83959 actions. The present lack of reversal effects with the administration of DAR antagonists may also be further investigated with microinjection experiments that specifically infuse the antagonists to contained brain regions with selective DARs of interest. Such experimental designs will help to elucidate the relationship between drug treatments and their target brain regions. The addition of control groups that solely received the drug treatments or the behavioral training will help to better dissociate whether the changes in brain protein expression came from biochemical effects of the drugs or processes of neural plasticity induced by long-term behavioral training. Moreover, co-immunoprecipitation experiments may provide further evidence on the presence and distribution of D_1-D_2 receptor heteromers in the brain. Finally, the dosage of SKF 83959 used in the current experiments may be too high for testing operant performance that leads to the absence of observed effects for pharmacological antagonism. The consideration of giving lower doses of SKF 83959 (eg. 0.75 mg/kg) to maintain a smaller but still significant effect on operant responding may yield in behavioral deficits that can be reversed by DAR antagonists. Also, the administration of SKF 83959 at lower doses may prevent the present observations of greatly diminished response rates in some of the animals.

Conclusion

The present study tested the presumable activation of D_1-D_2 receptor heteromers via SKF 83959 administrations on schedule-controlled operant behaviors using the FI 30-s and DRL 10-s schedules of reinforcement. SKF 83959 was found to reduce the response rates on both schedules, while the peak time on the DRL 10-s schedule was unaffected. In addition, the locomotor activity was not affected by SKF 83959, which suggested that the basic motor ability was not impaired by the drug. The effects of SKF 83959 on operant behaviors were not successfully reversed by the sole or combined pretreatments of SCH 23390 and eticlopride. Biochemically, SKF 83959 induced changes in the levels of pCaMKII, PKA, and pCREB, most prominently in the DS and NAc. It is hereby proposed that SKF 83959 affected the striatal functions that regulate reward-related motivation via the induction of changes in pCaMKII, PKA, and CREB signalling. The unsuccessful attempt to reverse the SKF 83959-induced behavioral effects may be attributed to the complexity of the biochemical system *in vivo*. At the same time, a thorough examination of the relationship between SKF 83959 and D_1-D_2 receptor heteromers *in vivo* in the future will help to better elucidate the functions of D_1-D_2 receptor heteromer activation.

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Table 1: *An outline of non-responsive rats after SKF 83959 administration*

Experiment	Task	Treatment	Total Number of Non-Responsive Subjects (Zero Lever Presses)	Subject #
1	FI 30-s (n = 12)	1.0 mg/kg SKF 83959	3	15, 17, 20, 21, 23
	DRL 10-s (n = 11)	1.0 mg/kg SKF 83959	1	3
2	FI 30-s (n = 6)	1.0 mg/kg SKF 83959	1	15
	DRL 10-s (n = 6)	1.0 mg/kg SKF 83959	1	1
4	FI 30-s (n = 8)	1.0 mg/kg SKF 83959 (First)	2	15, 18
		0.02 mg/kg SCH 23390 + 1.0 mg/kg SKF 83959	1	12
		1.0 mg/kg SKF 83959 (Second)	2	7, 12
		0.06 mg/kg SCH 23390 + 1.0 mg/kg SKF 83959	4	7, 9, 12, 15
	FI 30-s (n = 9)	1.0 mg/kg SKF 83959 (First)	3	15, 17, 18
		0.02 mg/kg Eticlopride + 1.0 mg/kg SKF 83959	2	17, 18
		1.0 mg/kg SKF 83959 (Second)	3	12, 15, 17
		0.06 mg/kg Eticlopride + 1.0 mg/kg SKF 83959	2	15, 18
		1.0 mg/kg SKF 83959 (Third)	4	14, 15, 16, 18
		0.02 mg/kg SCH 23390 + 0.02 mg/kg Eticlopride + 1.0 mg/kg SKF 83959	2	12, 17
	DRL 10-s (n = 9)	1.0 mg/kg SKF 83959 (First)	3	2, 4, 8
		0.02 mg/kg SCH 23390 + 1.0 mg/kg SKF 83959	4	2, 4, 8, 11
		1.0 mg/kg SKF 83959 (Second)	4	2, 5, 8, 11
		0.06 mg/kg SCH 23390 + 1.0 mg/kg SKF 83959	5	2, 4, 5, 8, 11
	DRL 10-s (n = 8)	1.0 mg/kg SKF 83959 (First)	1	3
		0.02 mg/kg Eticlopride + 1.0 mg/kg SKF 83959	1	3
		1.0 mg/kg SKF 83959 (Second)	3	1, 3, 9
		0.06 mg/kg Eticlopride + 1.0 mg/kg SKF 83959	4	3, 4, 6, 7
		1.0 mg/kg SKF 83959 (Third)	4	3, 4, 5, 7
		0.02 mg/kg SCH 23390 + 0.02 mg/kg Eticlopride + 1.0 mg/kg SKF 83959	5	1, 3, 4, 5, 9

Table 2: *Correlation between Levels of Phosphorylated Proteins in the PFC under FI 30-s* (* $p < 0.05$; ** $p < 0.01$; two-tailed; the index of total response is abbreviated as TR)

Correlations

Pearson Correlation	Drug	TR	PFC pCaMKII	PFC pERK	PFC PKA	PFC pCREB
Drug	1.000	-.838**	-.050	-.163	-.144	-.378
TR	-.838**	1.000	.069	-.108	.025	.180
PFC pCaMKII	-.050	.069	1.000	-.423	.792**	.507
PFC pERK	-.163	-.108	-.423	1.000	-.406	.029
PFC PKA	-.144	.025	.792**	-.406	1.000	.515
PFC pCREB	-.378	.180	.507	.029	.515	1.000

Table 3: *Correlation between Levels of Phosphorylated Proteins in the PFC under DRL 10-s* (* $p < 0.05$; ** $p < 0.01$; two-tailed; the index of total response is abbreviated as TR)

Correlations

Pearson Correlation	Drug	TR	PFC pCaMKII	PFC pERK	PFC PKA	PFC pCREB
Drug	1.000	-.674*	-.533	-.398	-.617*	.518
TR	-.674*	1.000	.120	.352	.237	-.280
PFC pCaMKII	-.533	.120	1.000	.098	.766**	.137
PFC pERK	-.398	.352	.098	1.000	.145	-.125
PFC PKA	-.617*	.237	.766**	.145	1.000	-.184
PFC pCREB	.518	-.280	.137	-.125	-.184	1.000

Table 4: *Correlation between Levels of Phosphorylated Proteins in the DS under FI 30-s*
(* $p < 0.05$; ** $p < 0.01$; two-tailed; the index of total response is abbreviated as TR)

Correlations

Pearson Correlation	Drug	TR	DS pCaMKII	DS pERK	DS PKA	DS pCREB
Drug	1.000	-.838**	-.502	.328	-.064	-.334
TR	-.838**	1.000	.462	-.295	-.052	.373
DS pCaMKII	-.502	.462	1.000	-.086	.739**	.702*
DS pERK	.328	-.295	-.086	1.000	-.104	-.128
DS PKA	-.064	-.052	.739**	-.104	1.000	.806**
DS pCREB	-.334	.373	.702*	-.128	.806**	1.000

Table 5: *Correlation between the Levels of Phosphorylated Proteins in the DS under DRL 10-s*
(* $p < 0.05$; ** $p < 0.01$; two-tailed; the index of total response is abbreviated as TR)

Correlations

Pearson Correlation	Drug	TR	DS pCaMKII	DS pERK	DS PKA	DS pCREB
Drug	1.000	-.674*	-.323	-.523	-.111	.093
TR	-.674*	1.000	.260	.491	.321	-.115
DS pCaMKII	-.323	.260	1.000	.760**	.123	.725*
DS pERK	-.523	.491	.760**	1.000	.332	.387
DS PKA	-.111	.321	.123	.332	1.000	-.089
DS pCREB	.093	-.115	.725*	.387	-.089	1.000

Table 6: *Correlation between the Levels of Phosphorylated Proteins in the NAc under FI 30-s* (* $p < 0.05$; ** $p < 0.01$; two-tailed; the index of total response is abbreviated as TR)

Correlations

Pearson Correlation	Drug	TR	NAc pCaMKII	NAc pERK	NAc PKA	NAc pCREB
Drug	1.000	-.838**	-.670*	-.151	-.624*	-.631*
TR	-.838**	1.000	.640*	.083	.502	.634*
Nac pCaMKII	-.670*	.640*	1.000	-.168	.402	.690*
NAc pERK	-.151	.083	-.168	1.000	-.212	.029
NAc PKA	-.624*	.502	.402	-.212	1.000	.042
NAc pCREB	-.631*	.634*	.690*	.029	.042	1.000

Table 7: *Correlation between the Levels of Phosphorylated Proteins in the NAc under DRL 10-s* (* $p < 0.05$; ** $p < 0.01$; two-tailed; the index of total response is abbreviated as TR)

Correlations

Pearson Correlation	Drug	TR	NAc pCaMKII	NAc pERK	NAc PKA	NAc pCREB
Drug	1.000	-.674*	.481	-.165	.538	.780**
TR	-.674*	1.000	-.090	.369	-.071	-.350
NAc pCaMKII	.481	-.090	1.000	-.344	.707*	.833**
NAc pERK	-.165	.369	-.344	1.000	-.154	-.338
NAc PKA	.538	-.071	.707*	-.154	1.000	.753**
NAc pCREB	.780**	-.350	.833**	-.338	.753**	1.000

Table 8: Correlation between the Levels of Phosphorylated Proteins in the Hippocampus under FI 30-s (* p < 0.05; ** p < 0.01; two-tailed; the index of total response is abbreviated as TR)

Correlations

Pearson Correlation	Drug	TR	HIPPO pCaMKII	HIPPO pERK	HIPPO PKA	HIPPO pCREB
Drug	1.000	-.838**	-.095	-.165	.476	-.810**
TR	-.838**	1.000	.173	.249	-.221	.683*
HIPPO pCaMKII	-.095	.173	1.000	-.344	-.121	.097
HIPPO pERK	-.165	.249	-.344	1.000	.060	.160
HIPPO PKA	.476	-.221	-.121	.060	1.000	-.261
HIPPO pCREB	-.810**	.683*	.097	.160	-.261	1.000

Table 9: Correlation between the Levels of Phosphorylated Proteins in the Hippocampus under DRL 10-s(* p < 0.05; ** p < 0.01; two-tailed; the index of total response is abbreviated as TR)

Correlations

Pearson Correlation	Drug	TR	HIPPO pCaMKII	HIPPO pERK	HIPPO PKA	HIPPO pCREB
Drug	1.000	-.674*	-.117	-.317	-.194	.319
TR	-.674*	1.000	-.099	.163	.201	.093
HIPPO pCaMKII	-.117	-.099	1.000	.527	-.323	-.127
HIPPO pERK	-.317	.163	.527	1.000	-.443	-.021
HIPPO PKA	-.194	.201	-.323	-.443	1.000	-.280
HIPPO pCREB	.319	.093	-.127	-.021	-.280	1.000

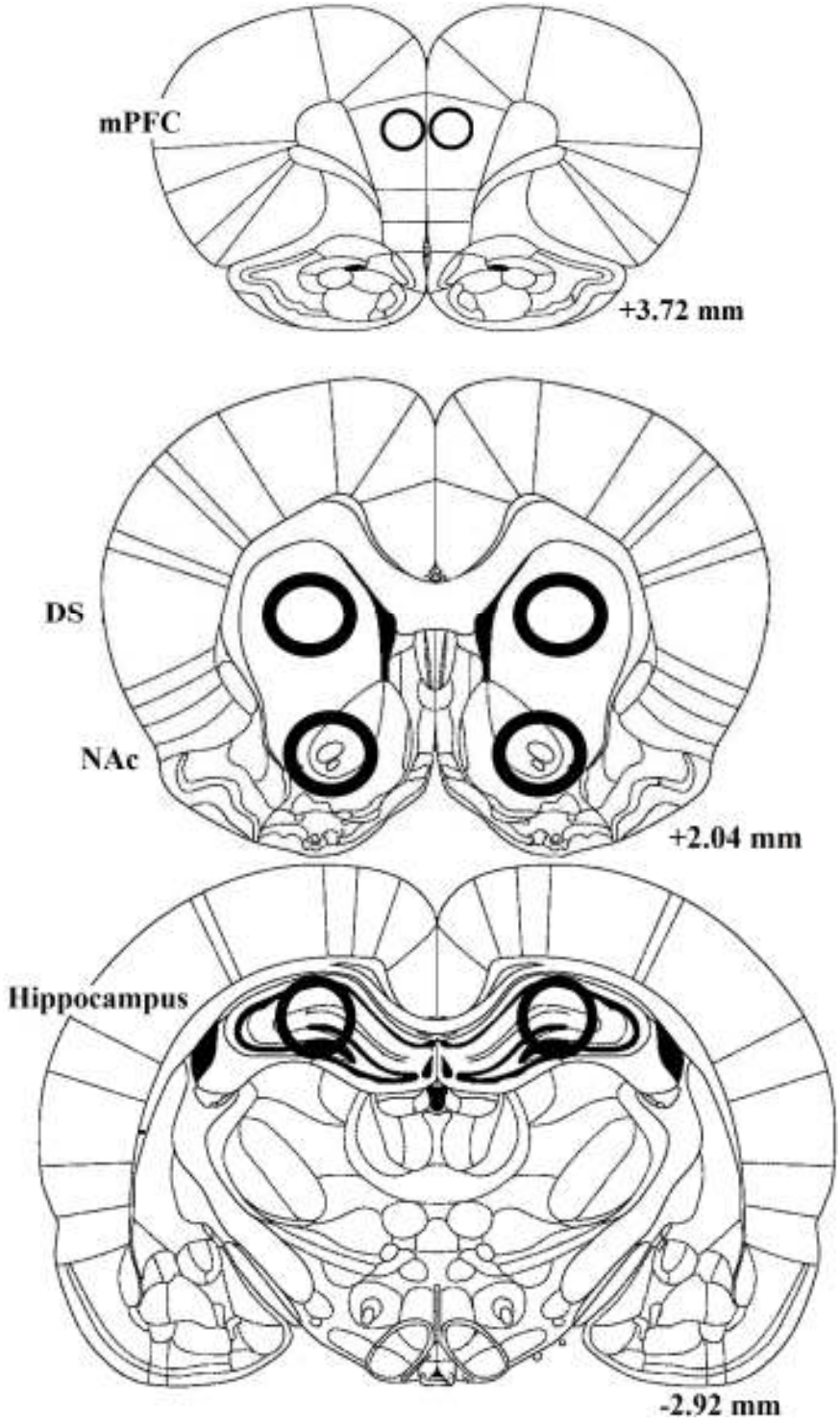

Figure 1. Schematic diagrams of the regions from where brain tissues were collected in Experiment 2: medial prefrontal cortex (mPFC), dorsal striatum (DS), nucleus accumbens (NAc), and dorsal hippocampus.

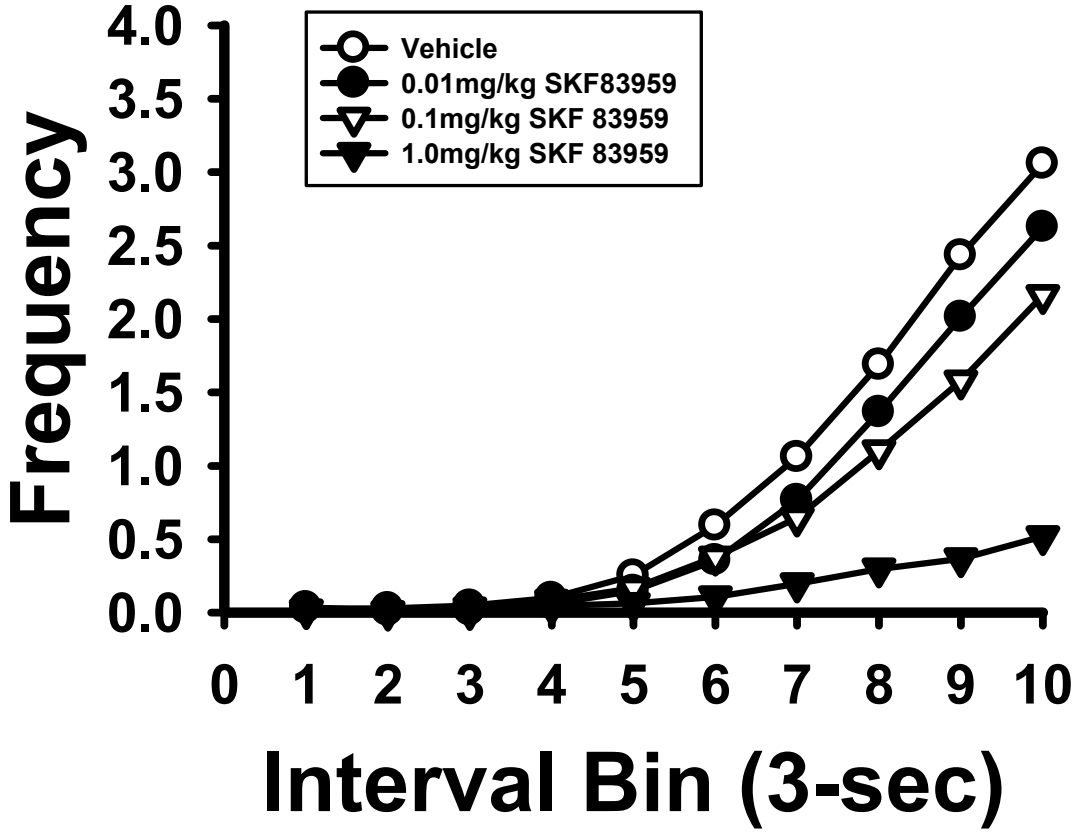

Figure 2. The dose effects of SKF 83959 on the IRT curve of FI 30-s performance in a within-subjects design (n = 12). Rats were injected with SKF 83959 at four doses on consecutive days. SKF 83959 appeared to dose-dependently reduce the response frequency. (Exp.1)

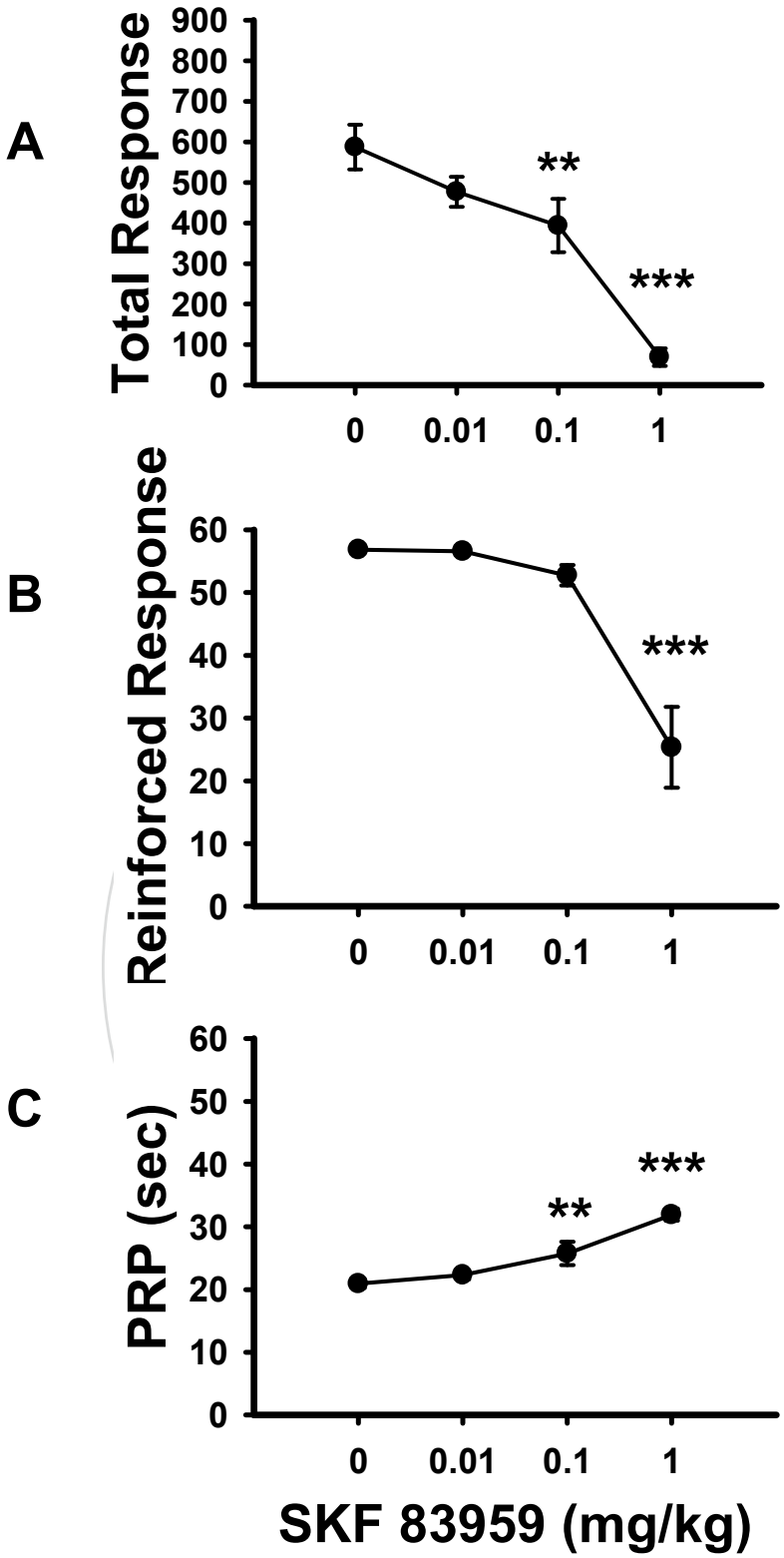

Figure 3. The dose effects of SKF 83959 on FI 30-s response-based indexes (A, B) and PRP (C). The rats treated with moderate and high doses of SKF 83959 showed reduced response rates and increased PRP durations. ** $p < 0.01$; *** $p < 0.001$ compared to vehicle-treated group. (Exp.1)

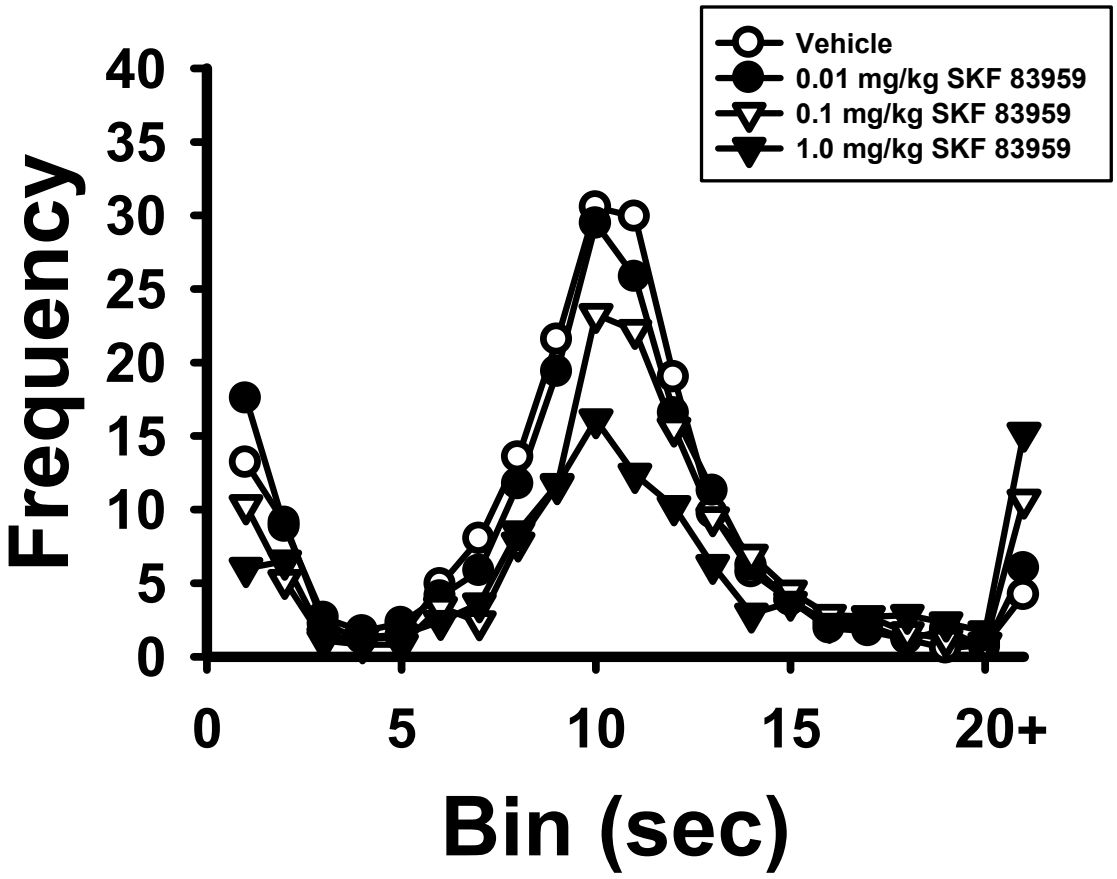

Figure 4. The dose effects of SKF 83959 on the IRT curve of DRL 10-s performance in a within-subjects design (n = 11). Rats were injected with SKF 83959 at four doses on consecutive days. SKF 83959 appeared to dose-dependently reduce the response frequency without producing any horizontal shifts in peak time. (Exp.1)

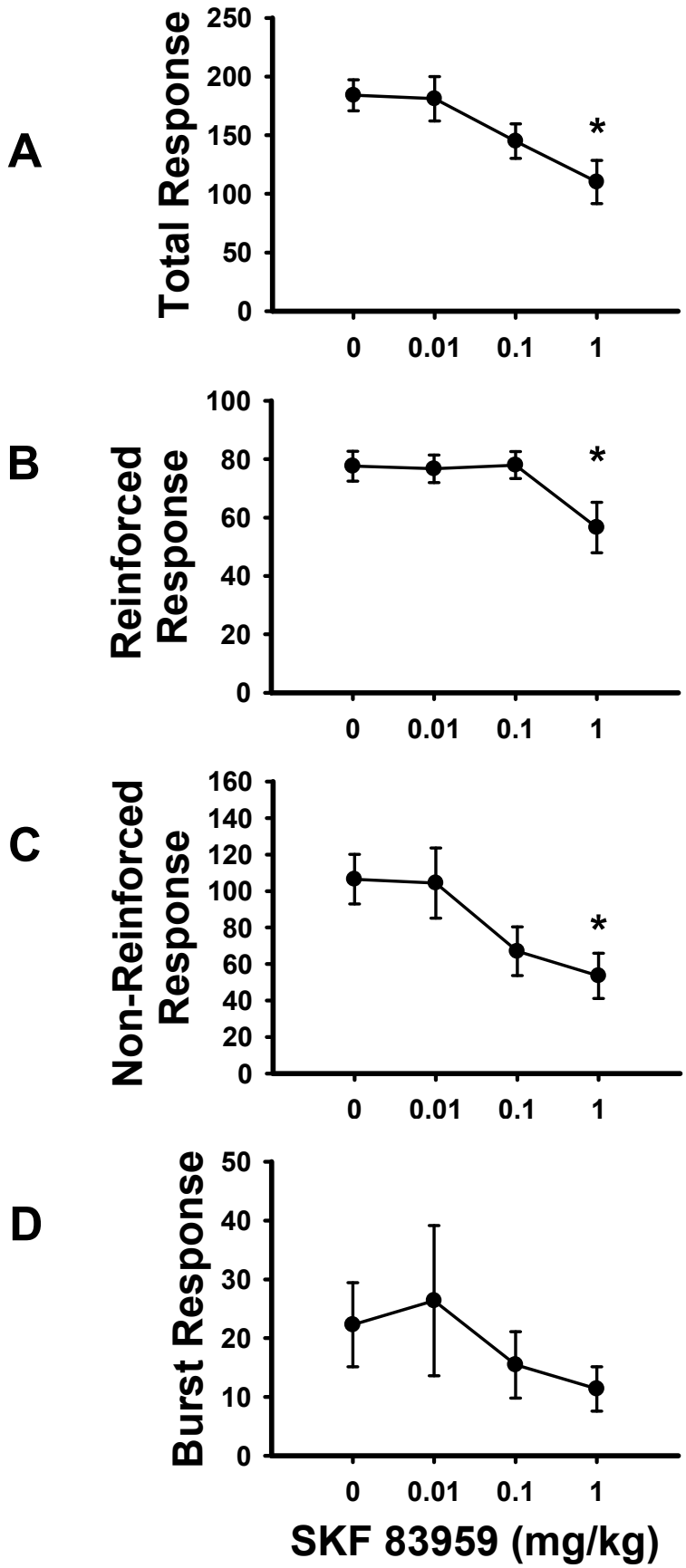

Figure 5. The dose effects of SKF 83959 on DRL 10-s response-based indexes. The rats under high dose SKF 83959 showed reduced response rates. * $p < 0.05$ compared to vehicle-treated group. (Exp.1)

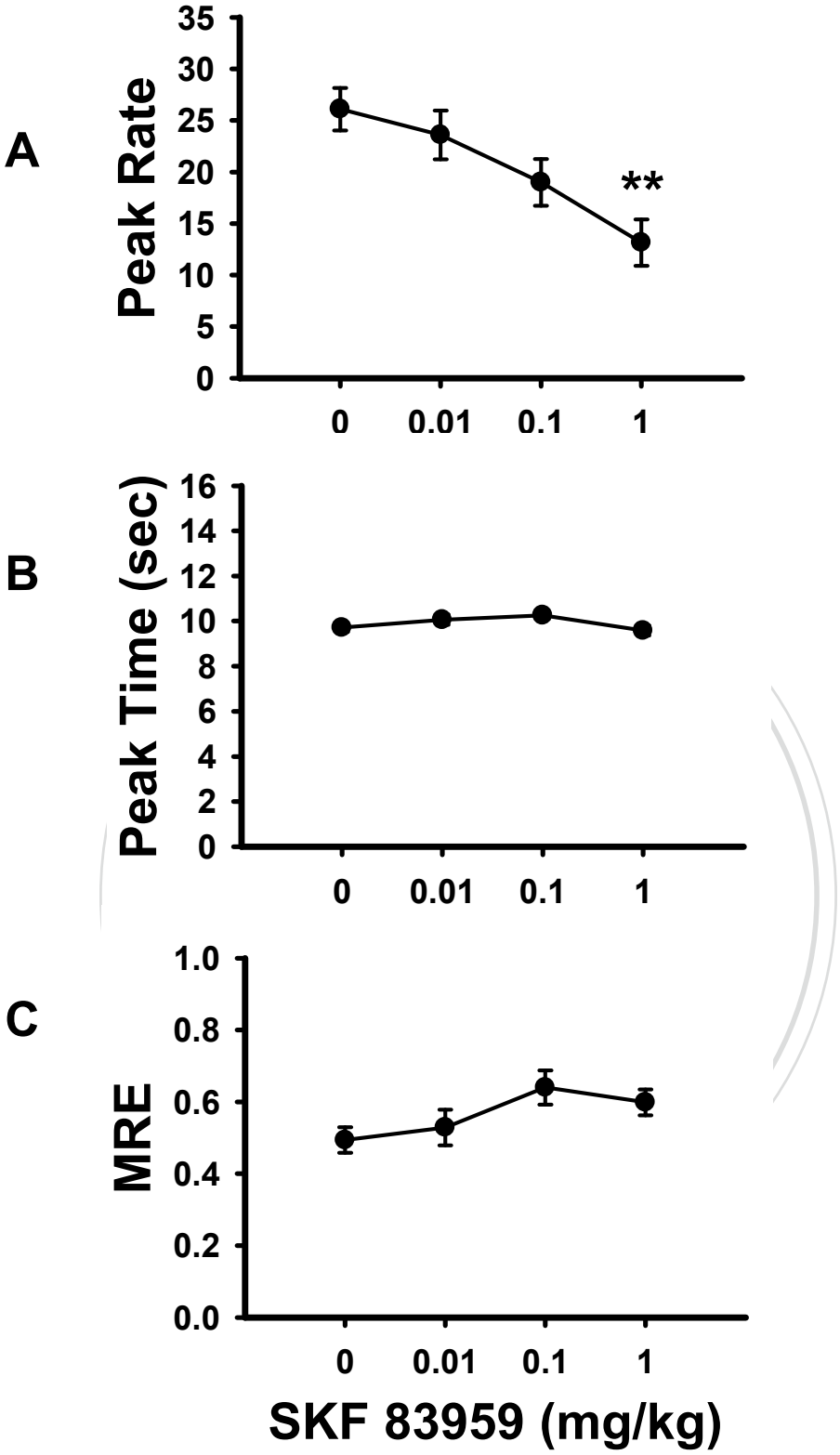

Figure 6. The dose effects of SKF 83959 on DRL 10-s peak rate (A), peak time (B), MRE (C). The rats under high dose SKF 83959 showed reduced peak rates, but the peak time was not affected by the drug. ** $p < 0.01$ compared to vehicle-treated group. (Exp.1)

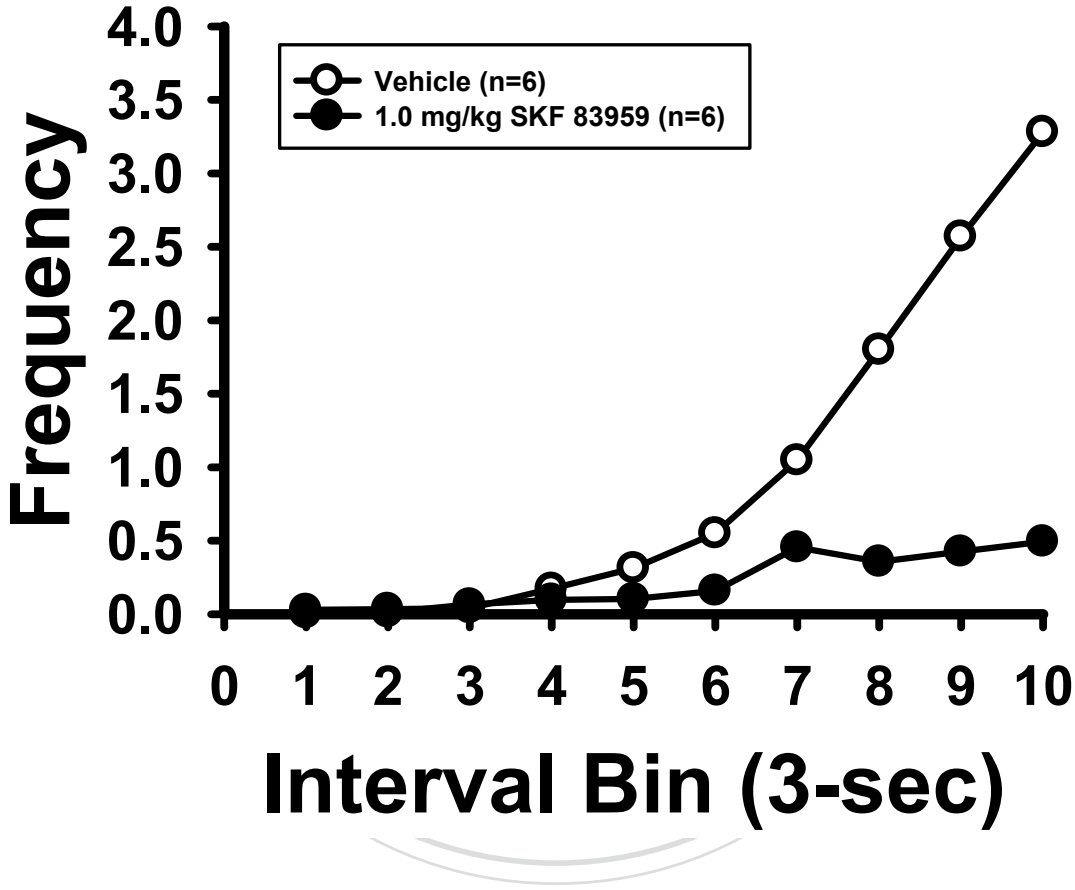

Figure 7. The effects of 1.0 mg/kg SKF 83959 on the IRT curve of FI 30-s performance in a between-subjects design. SKF 83959 at 1.0 mg/kg reduced the response frequency in a similar pattern as that in Experiment 1. (Exp.2)

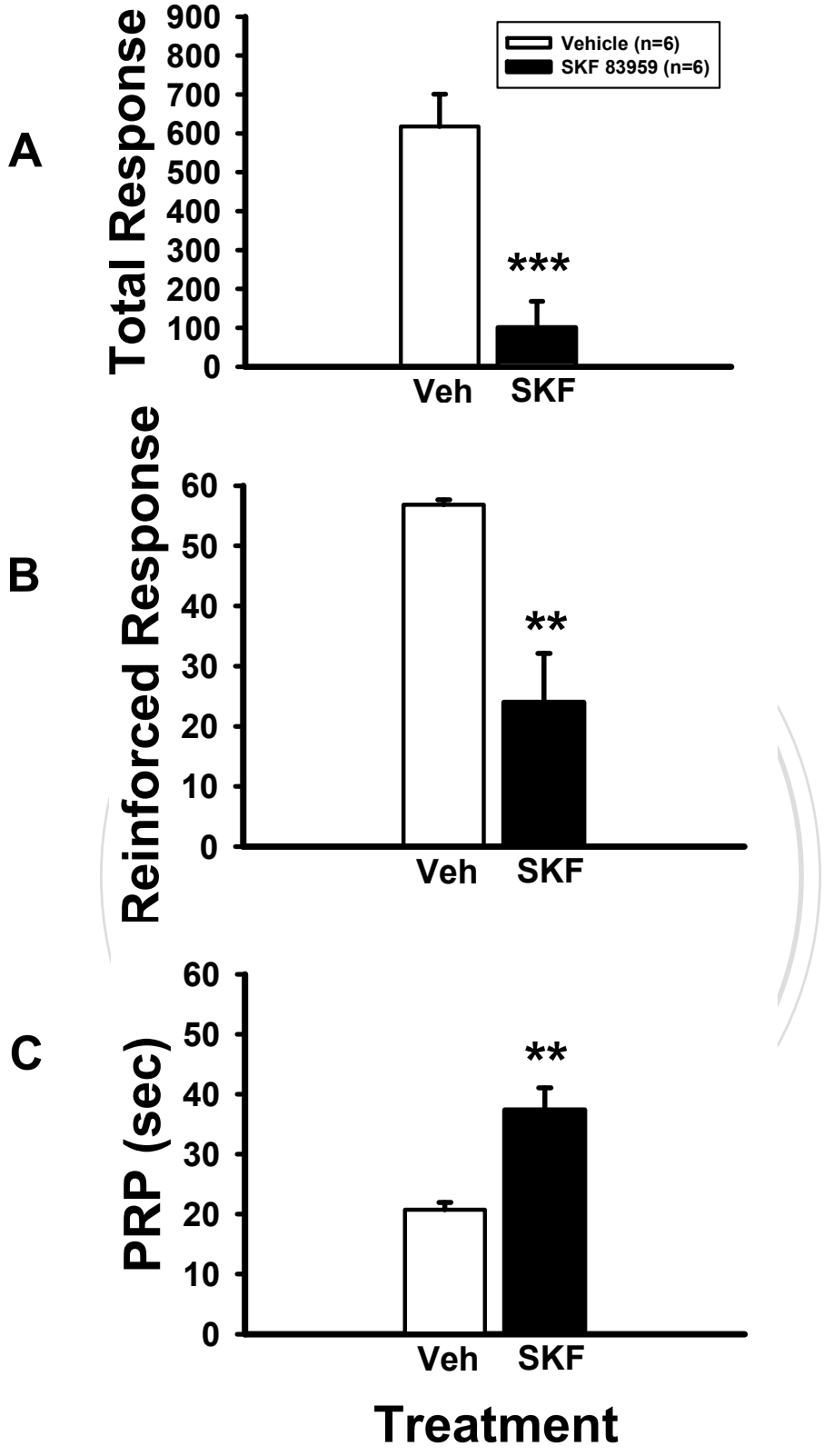

Figure 8. The effects of 1.0 mg/kg SKF 83959 on FI 30-s response-based indexes (A, B) and PRP (C). The drug-treated rats showed reduced response rates and increased PRP. ** $p < 0.01$; *** $p < 0.001$ compared to the vehicle-treated group. (Exp.2)

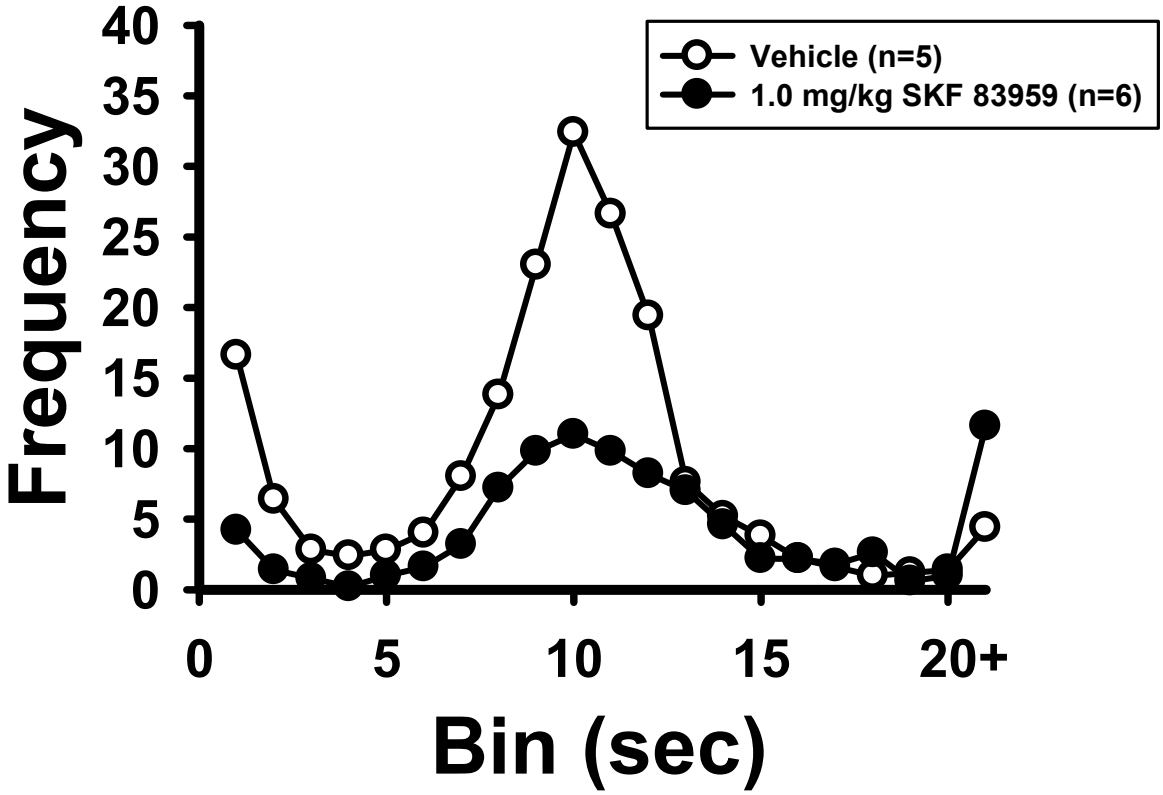

Figure 9. The effects of 1.0 mg/kg SKF 83959 on the IRT curve of DRL 10-s performance in a between-subjects design. SKF 83959 at 1.0 mg/kg reduced the response frequency without producing any horizontal shifts in peak time, in a similar pattern as that in Experiment 1. (Exp.2)

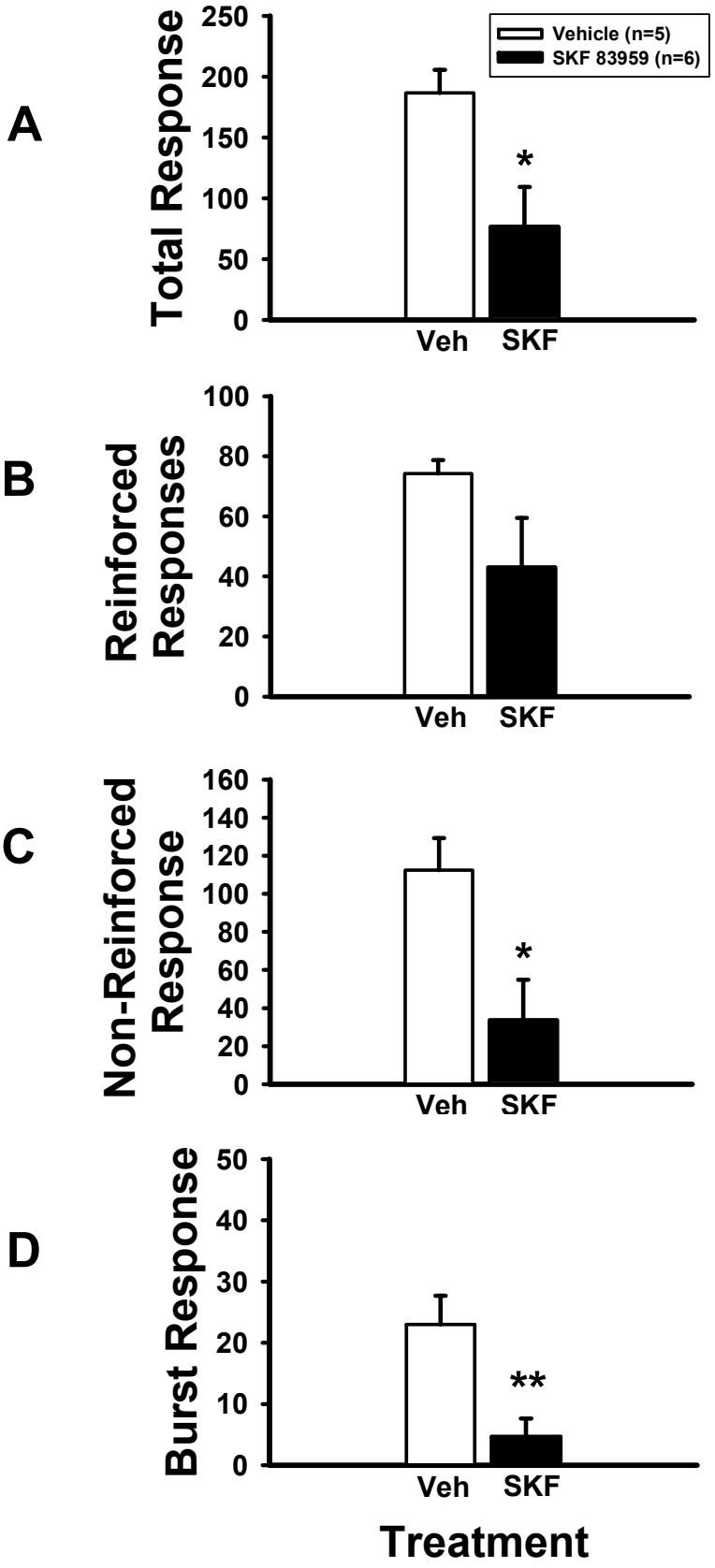

Figure 10. The effects 1.0 mg/kg SKF 83959 on DRL 10-s response-based indexes. The drug-treated rats showed reduced response rates. * $p < 0.05$; ** $p < 0.01$ compared to the vehicle-treated group. (Exp.2)

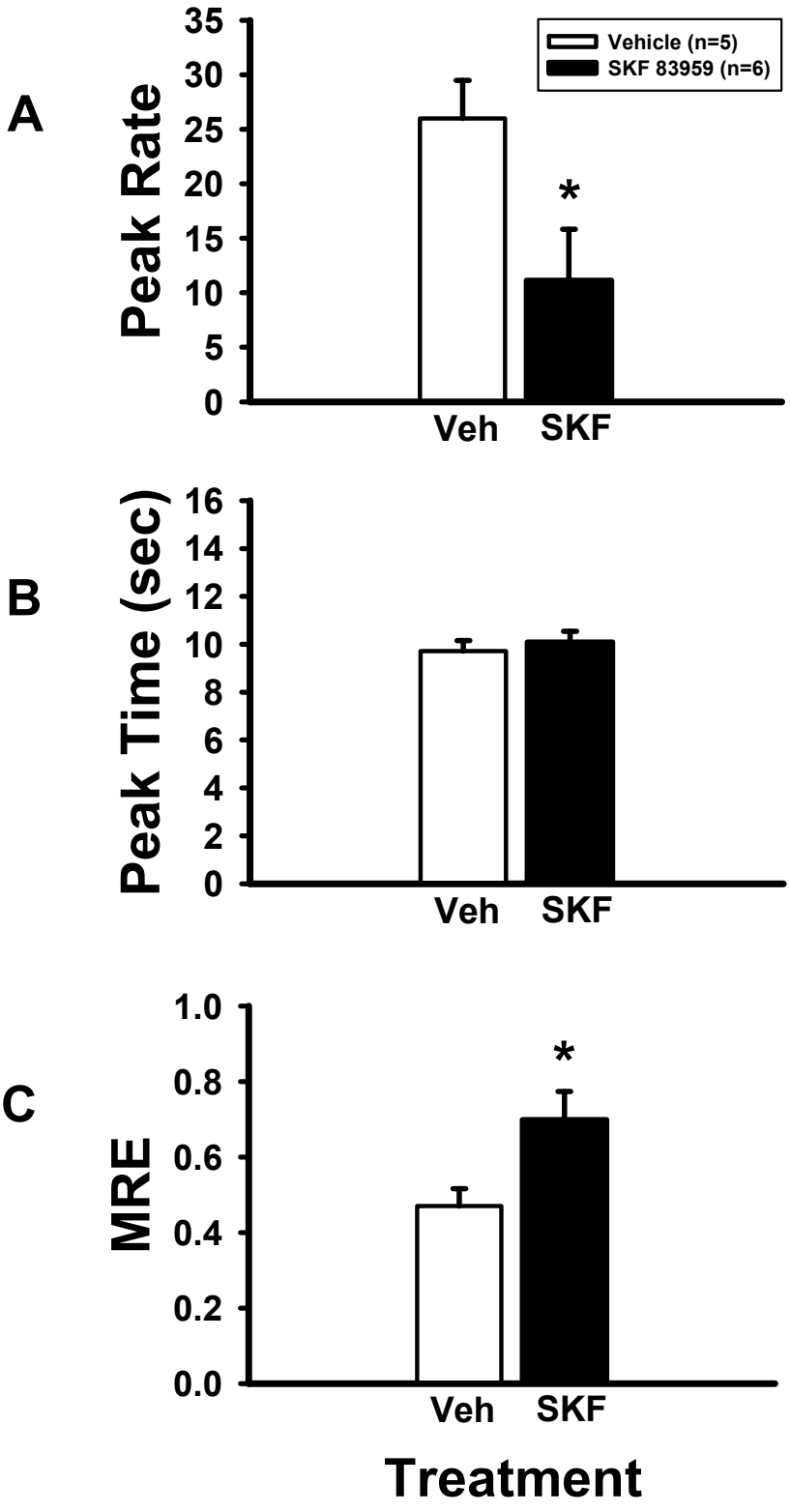

Figure 11. The effects of 1.0 mg/kg SKF 83959 on DRL 10-s peak rate (A), peak time (B), MRE (C). The drug-treated rats showed reduced peak rates, but the peak time was not affected.

* $p < 0.05$ compared to the vehicle-treated group. (Exp.2)

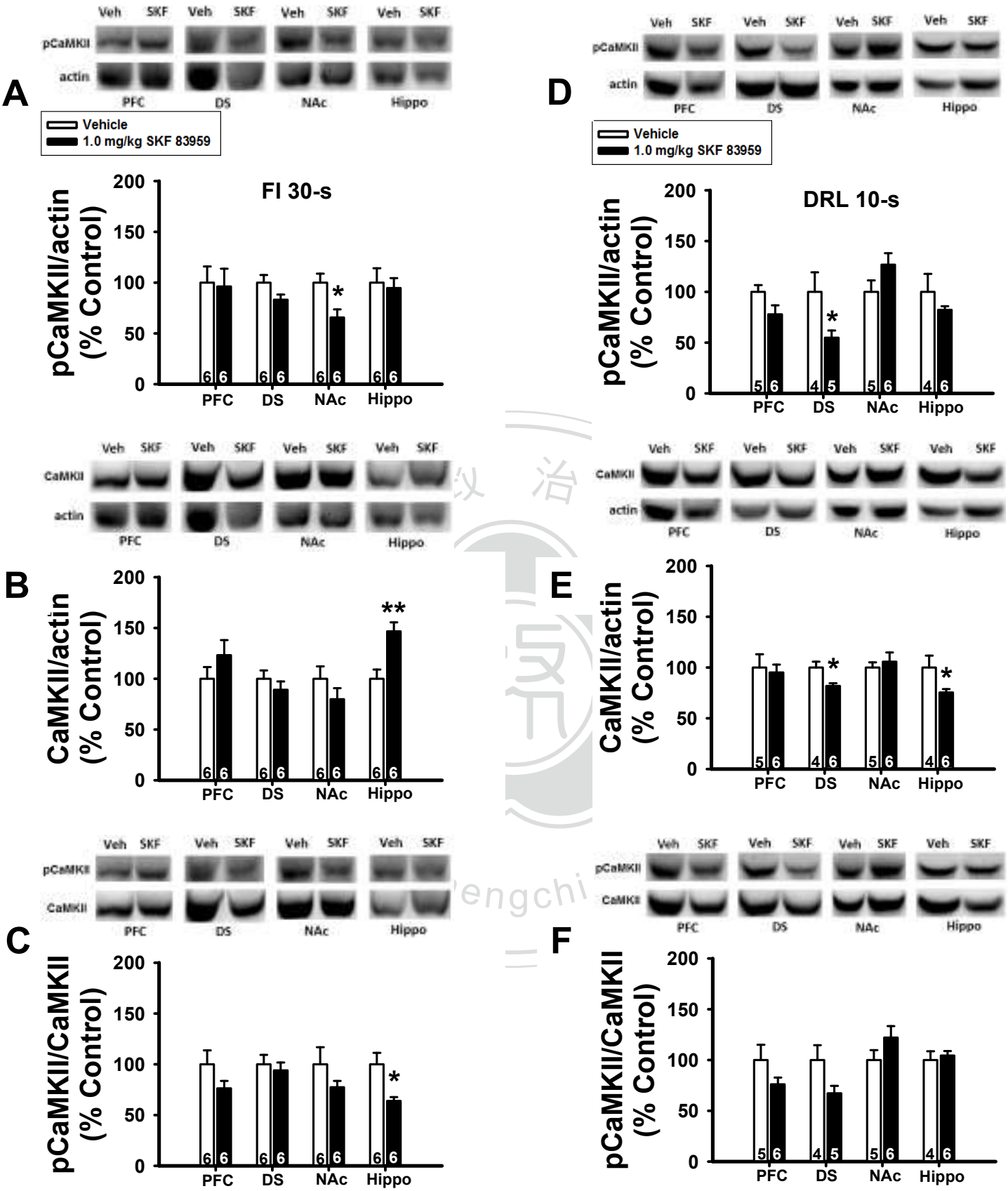

Figure 12. Levels of CaMKII expression in the specified brain regions after behavioral testing under FI 30-s (A, B, C) and DRL 10-s (D, E, F). * $p < 0.05$; ** $p < 0.01$ compared to the respective vehicle.

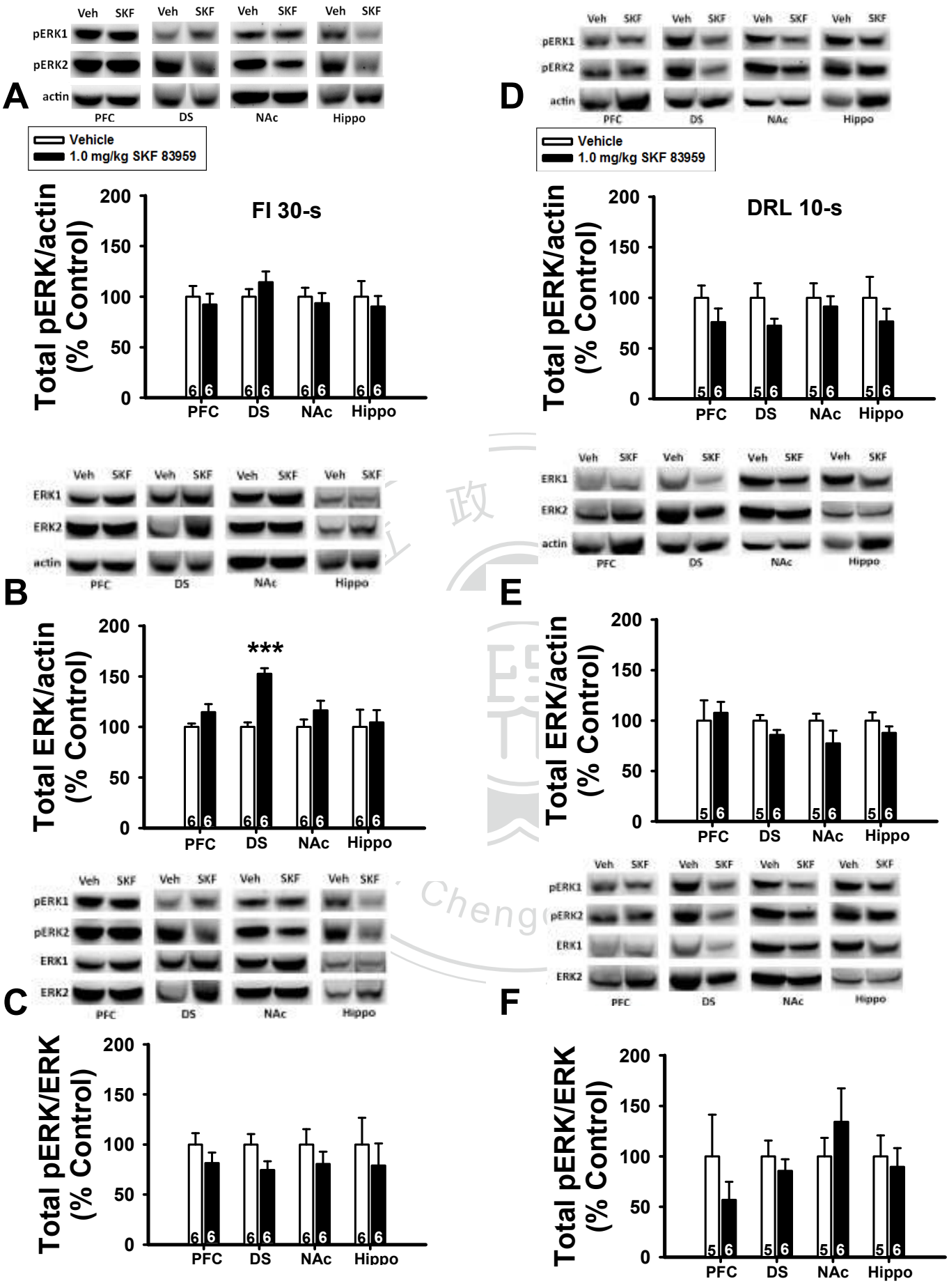

Figure 13. Levels of total ERK expression in the specified brain regions after behavioral testing under FI 30-s (A, B, C) and DRL 10-s (D, E, F).*** $p < 0.001$ compared to the respective vehicle.

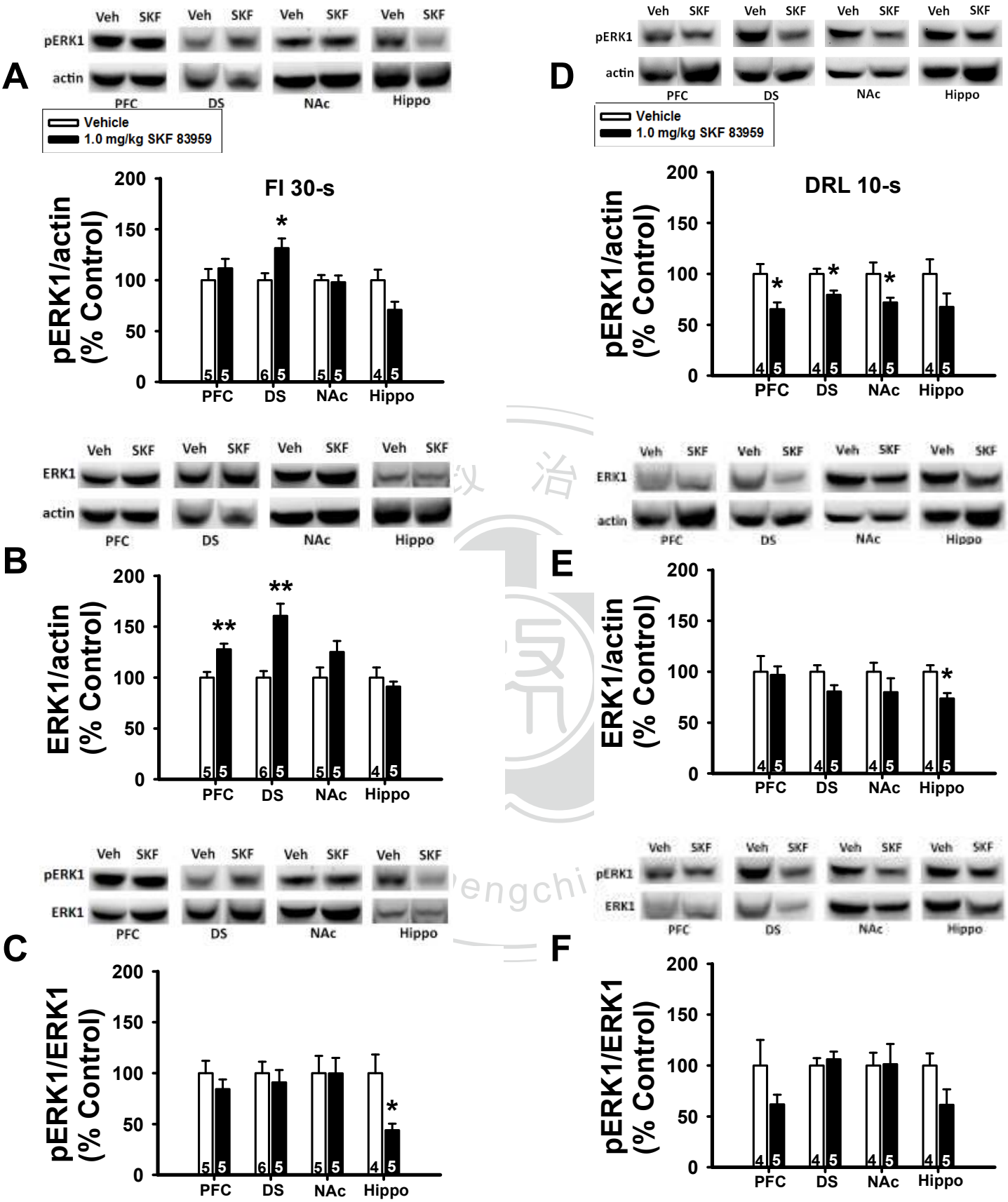

Figure 14. Levels of ERK1 expression in the specified brain regions after behavioral testing under FI 30-s (A, B, C) and DRL 10-s (D, E, F). * $p < 0.05$; ** $p < 0.01$ compared to respective vehicle.

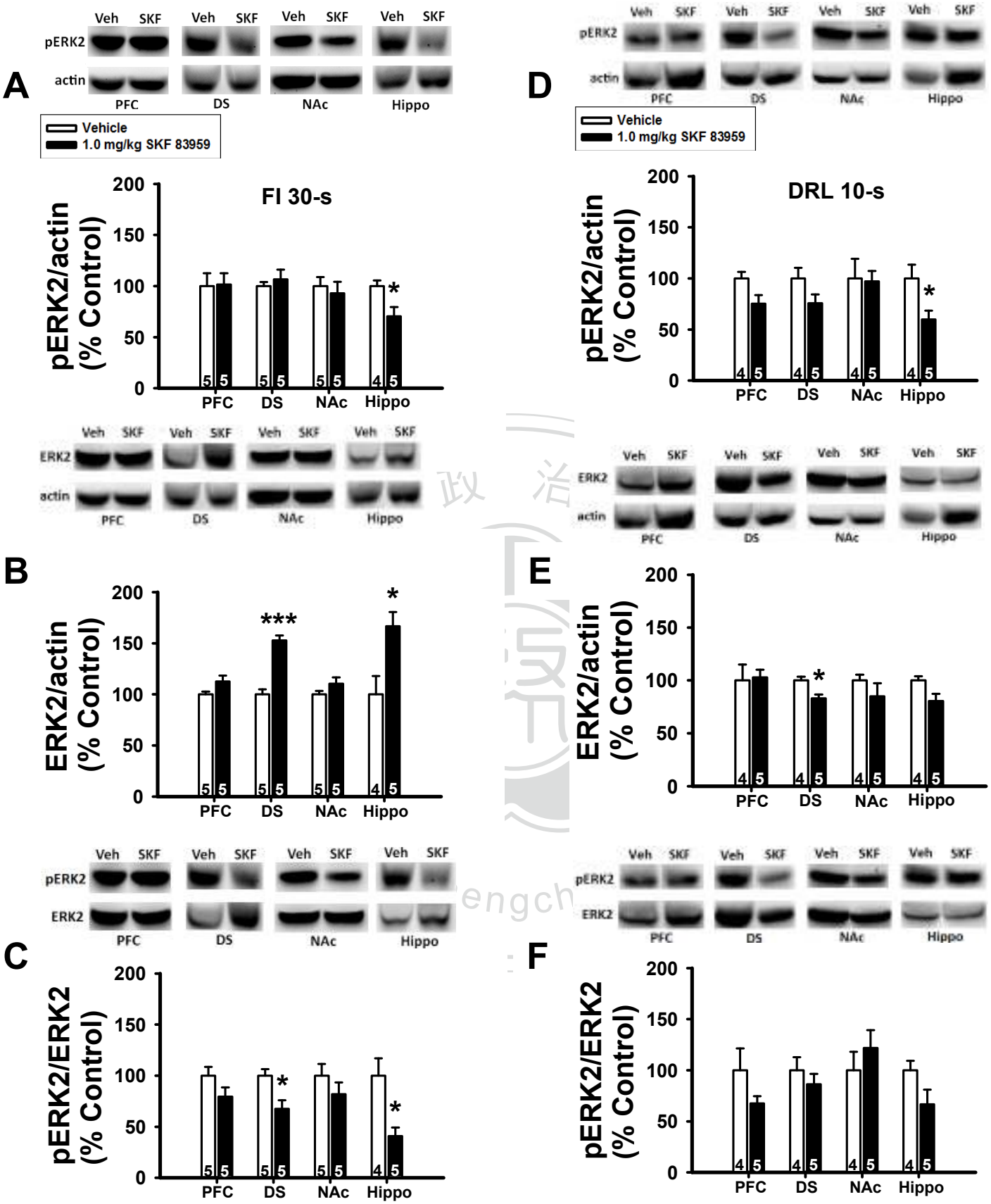

Figure 15. Levels of ERK2 expression in the specified brain regions after behavioral testing under FI 30-s (A, B, C) and DRL 10-s (D, E, F). * $p < 0.05$; *** $p < 0.001$ compared to respective vehicle.

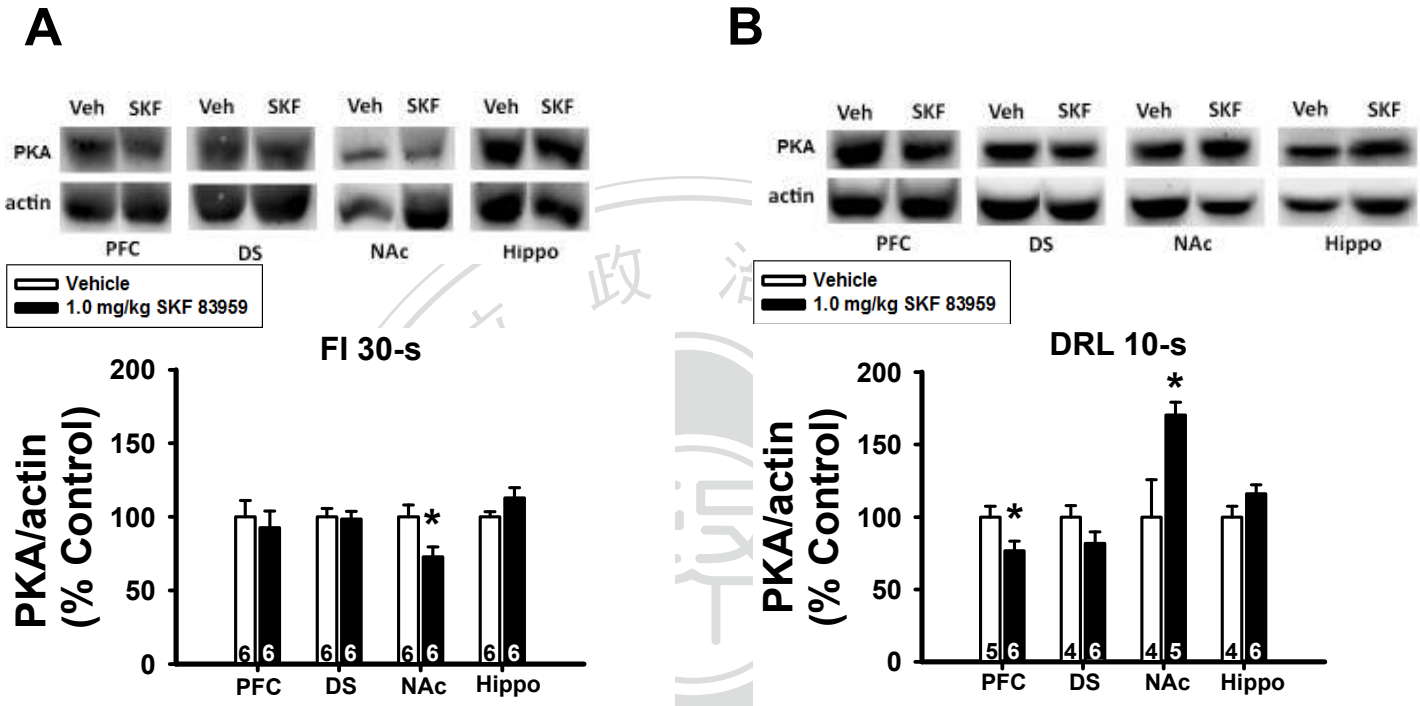

Figure 16. Levels of PKA expression in the specified brain regions after behavioral testing under FI 30-s (A) and DRL 10-s (B). * $p < 0.05$ compared to the respective vehicle.

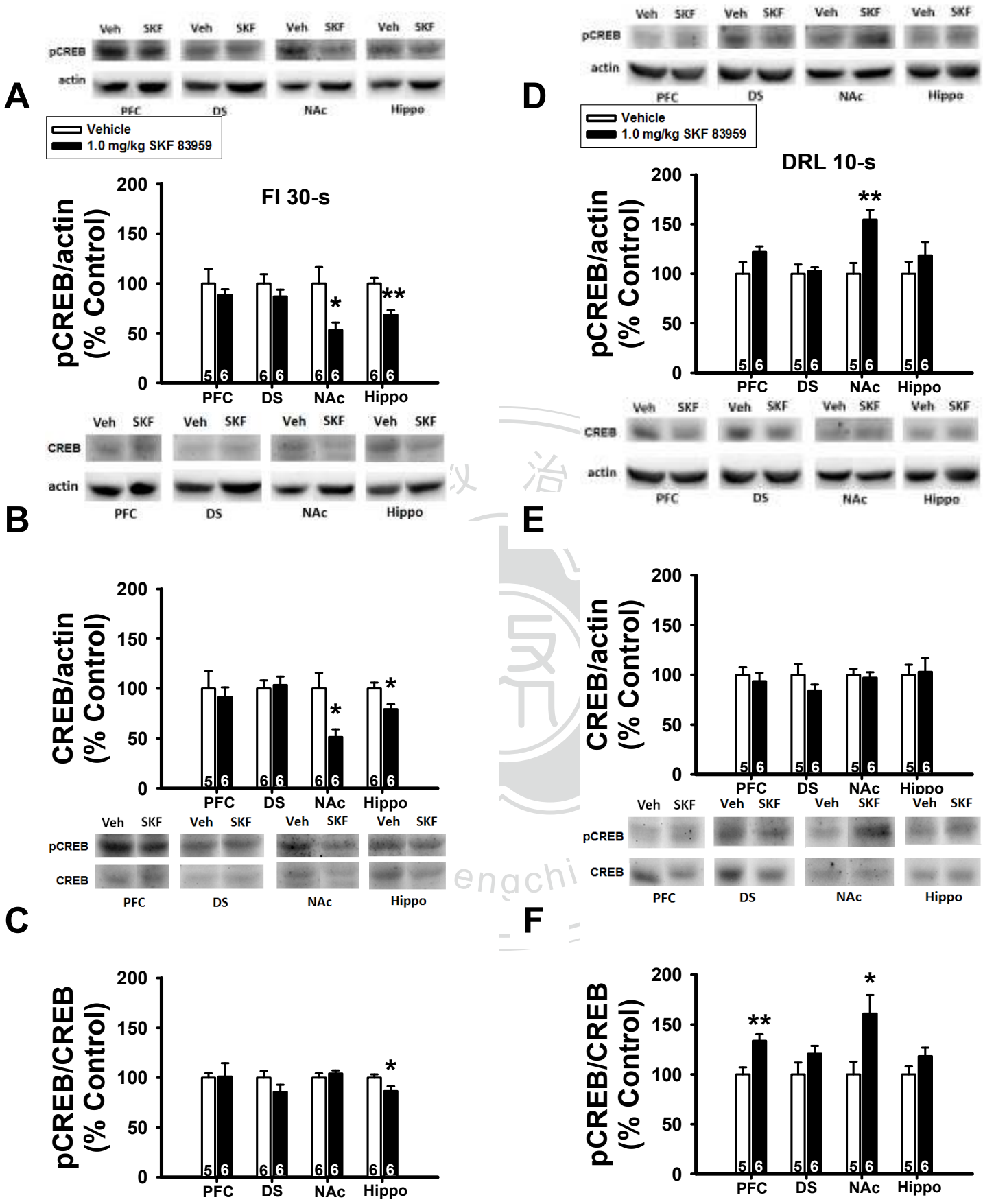

Figure 17. Levels of CREB expression in the specified brain regions after behavioral testing under FI 30-s (A, B, C) and DRL 10-s (D, E, F). * $p < 0.05$; ** $p < 0.01$ compared to respective vehicle.

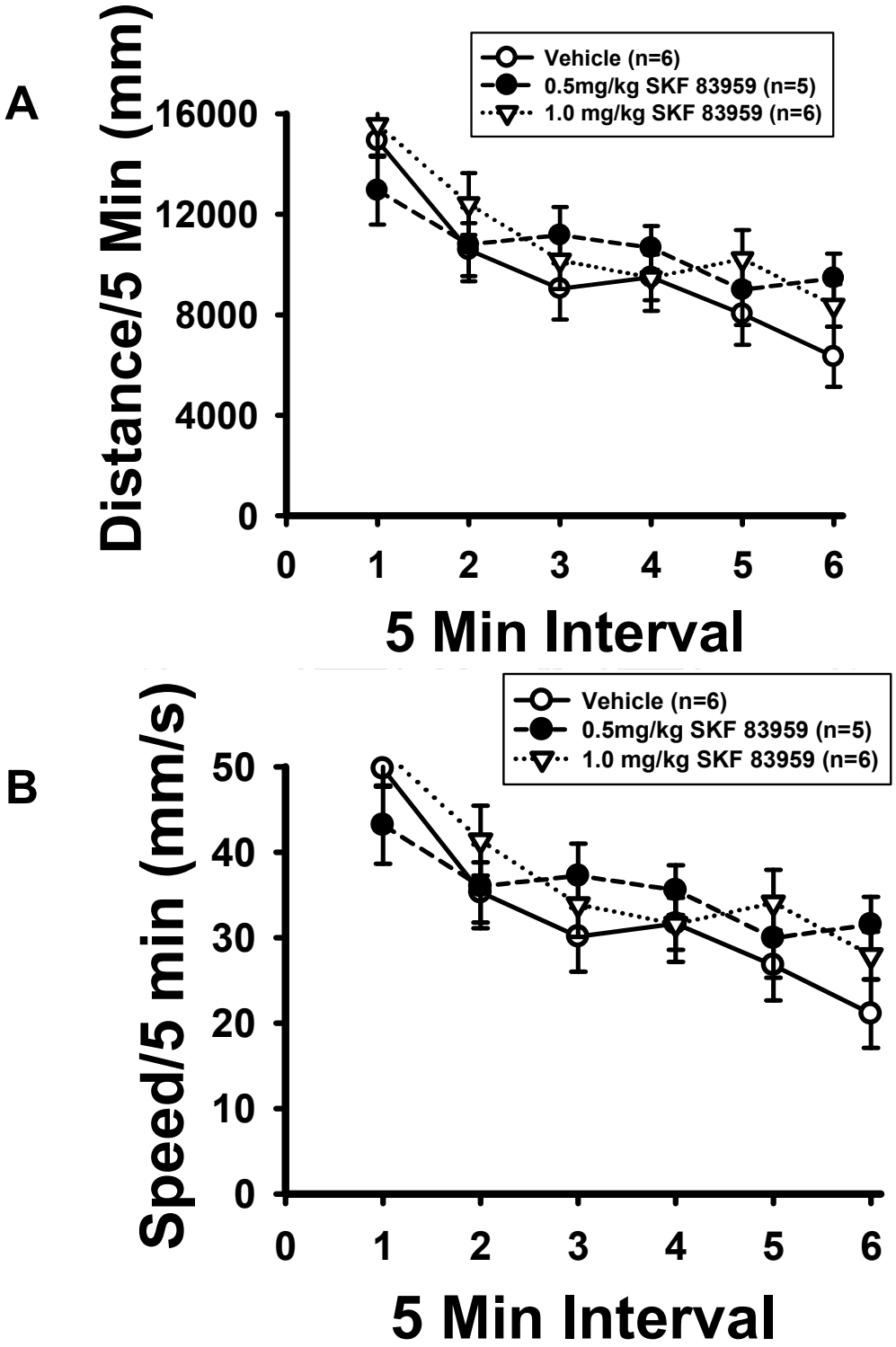

Figure 18. The dose effects of SKF 83959 on open-field locomotor activity: distance (A) and speed (B). Neither of the drug dose nor the drug dose and interval interaction had an effect on the locomotor activity; the locomotor activity was reduced by intervals due to habituation. (Exp.3)

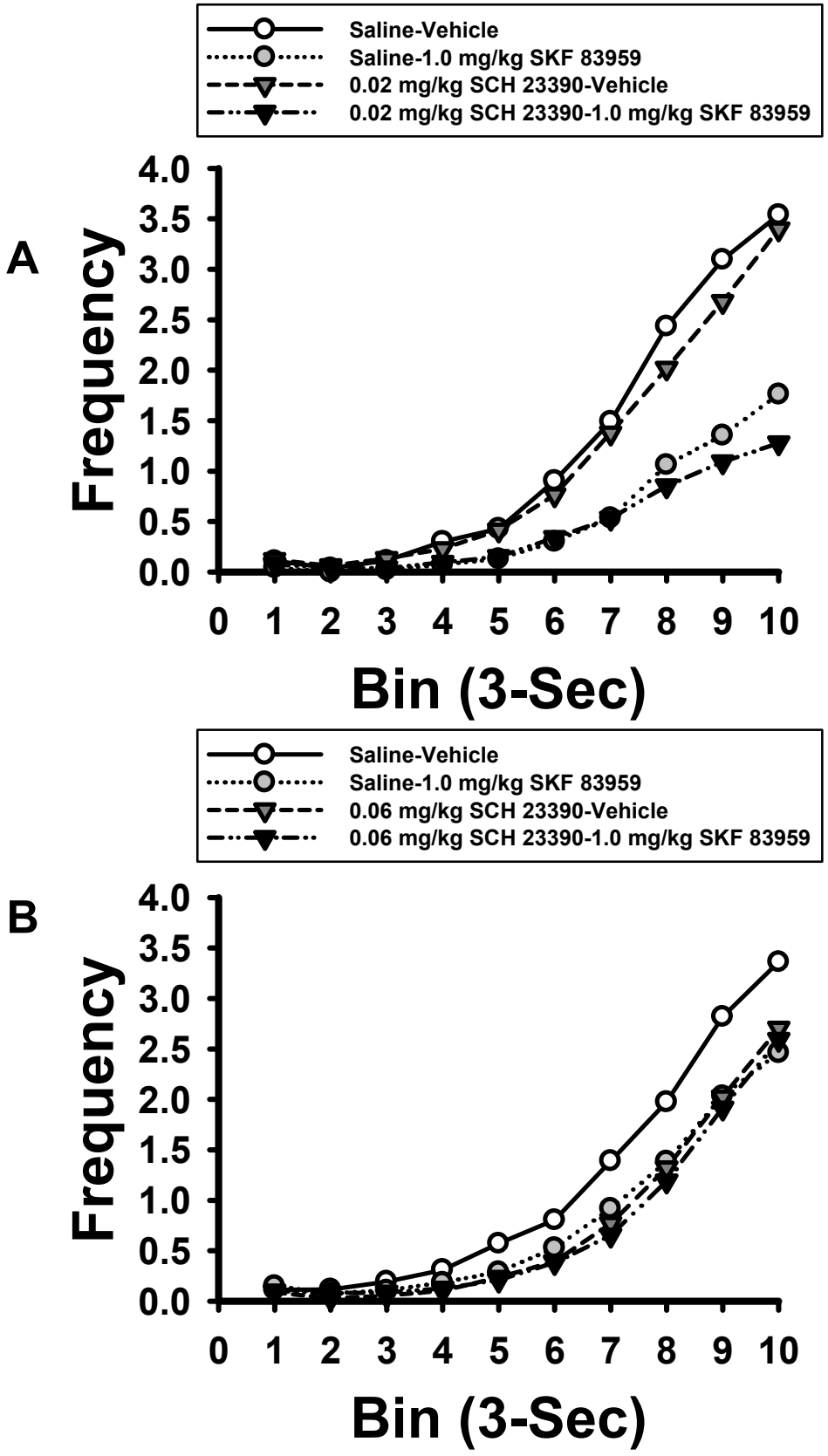

Figure 19. The FI 30-s IRT curve after SCH 23390 pretreatment in a within-subjects design (n = 8): 0.02 mg/kg SCH 23390 (A) and 0.06 mg/kg SCH 23390 (B). Neither doses of SCH 23390 appeared to reverse the SKF 83959-induced decline in response frequency on the FI 30-s schedule (Exp.4)

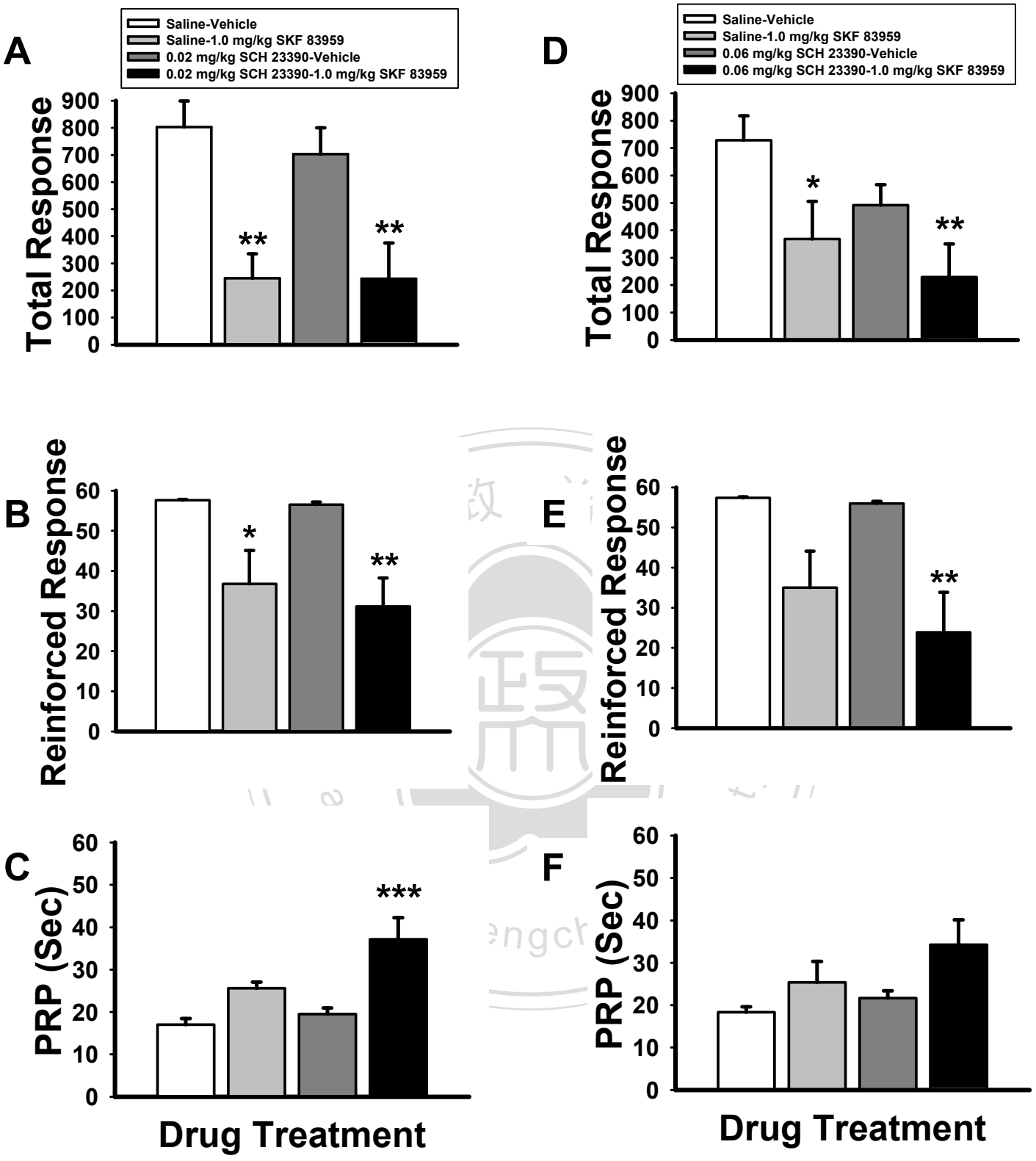

Figure 20. The FI 30-s indexes after SCH 23390 pretreatment (n = 8): 0.02 mg/kg SCH 23390 (A, B, C) and 0.06 mg/kg SCH 23390 (D, E, F). High dose SCH 23390 affected the total response rates to a greater extent than the low dose. However neither doses of SCH 23390 reversed the effects of SKF 83959 on the FI 30-s indexes. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ in comparison to the respective vehicle (Exp.4).

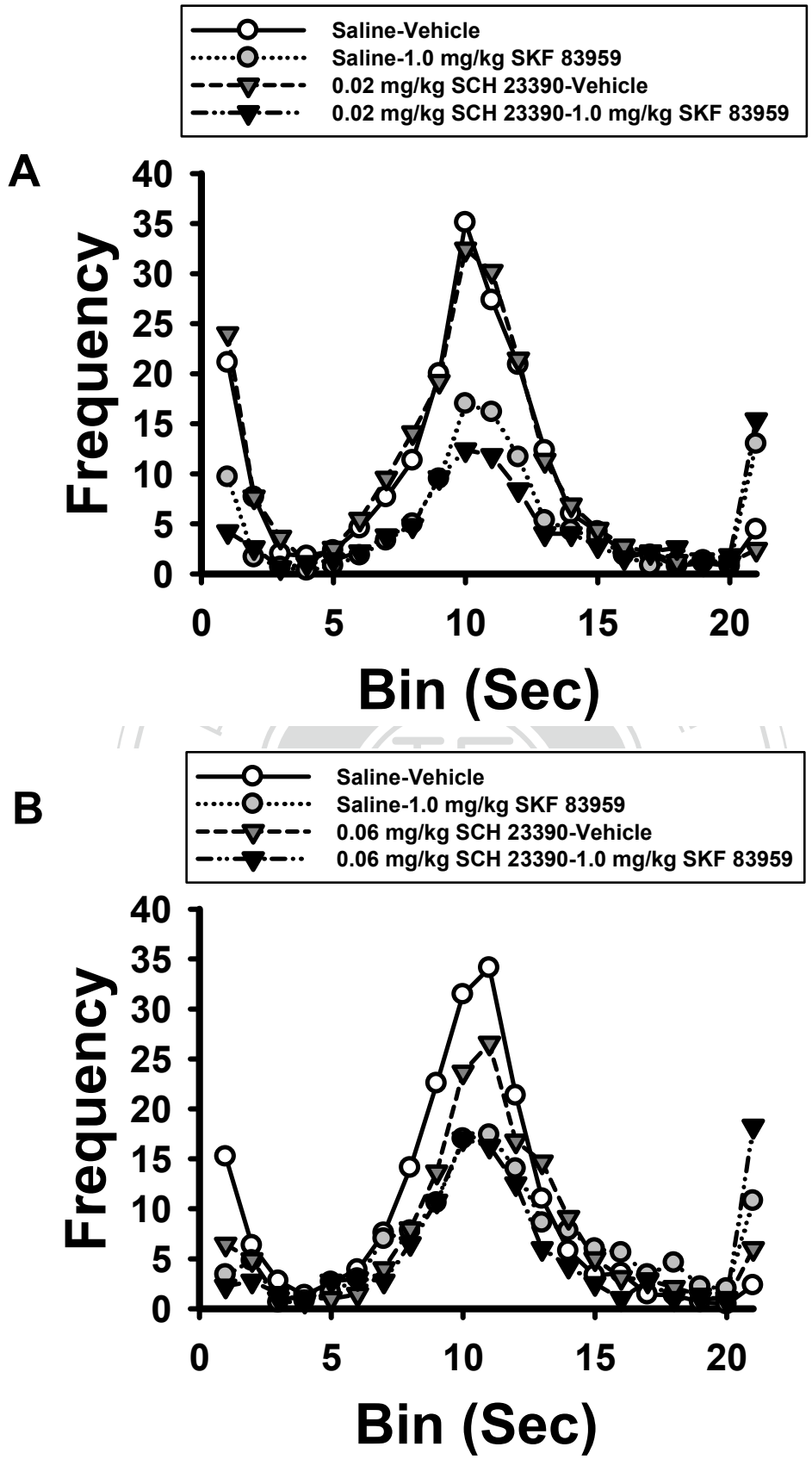

Figure 21. The DRL 10-s IRT curve after SCH 23390 pretreatment in a within-subjects design (n = 9): 0.02 mg/kg SCH 23390 (A) and 0.06 mg/kg SCH 23390 (B). Neither doses of SCH 23390 appeared to reverse the SKF 83959-induced decline in response frequency on the DRL 10-s schedule. (Exp.4)

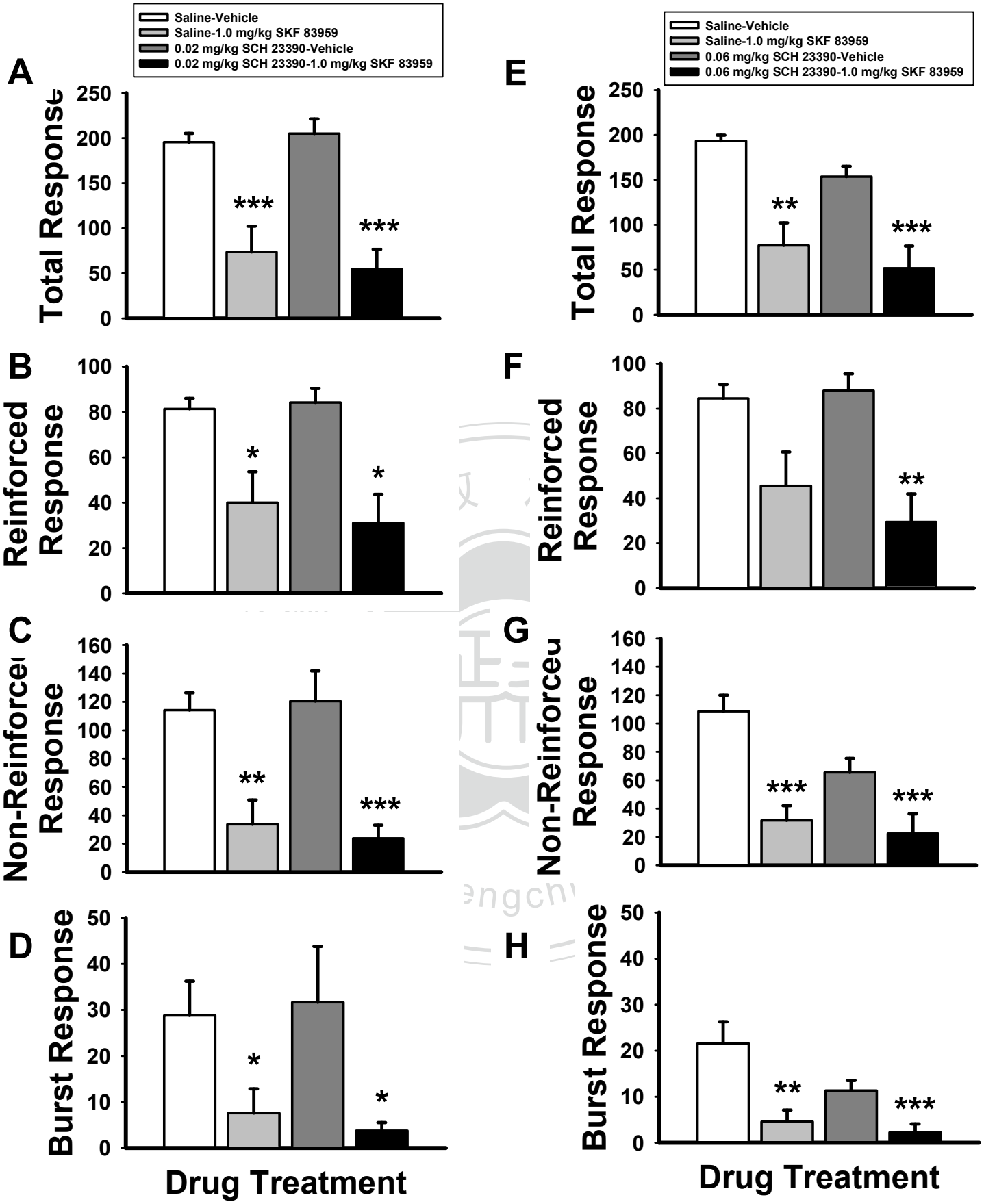

Figure 22. The DRL 10-s response-based indexes after SCH 23390 pretreatment (n = 9): 0.02 mg/kg SCH 23390 (A, B, C, D) and 0.06 mg/kg SCH 23390 (E, F, G, H). Neither doses of SCH 23390 reversed the effects of SKF 83959 on the DRL 10-s indexes. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ to vehicle. (Exp.4)

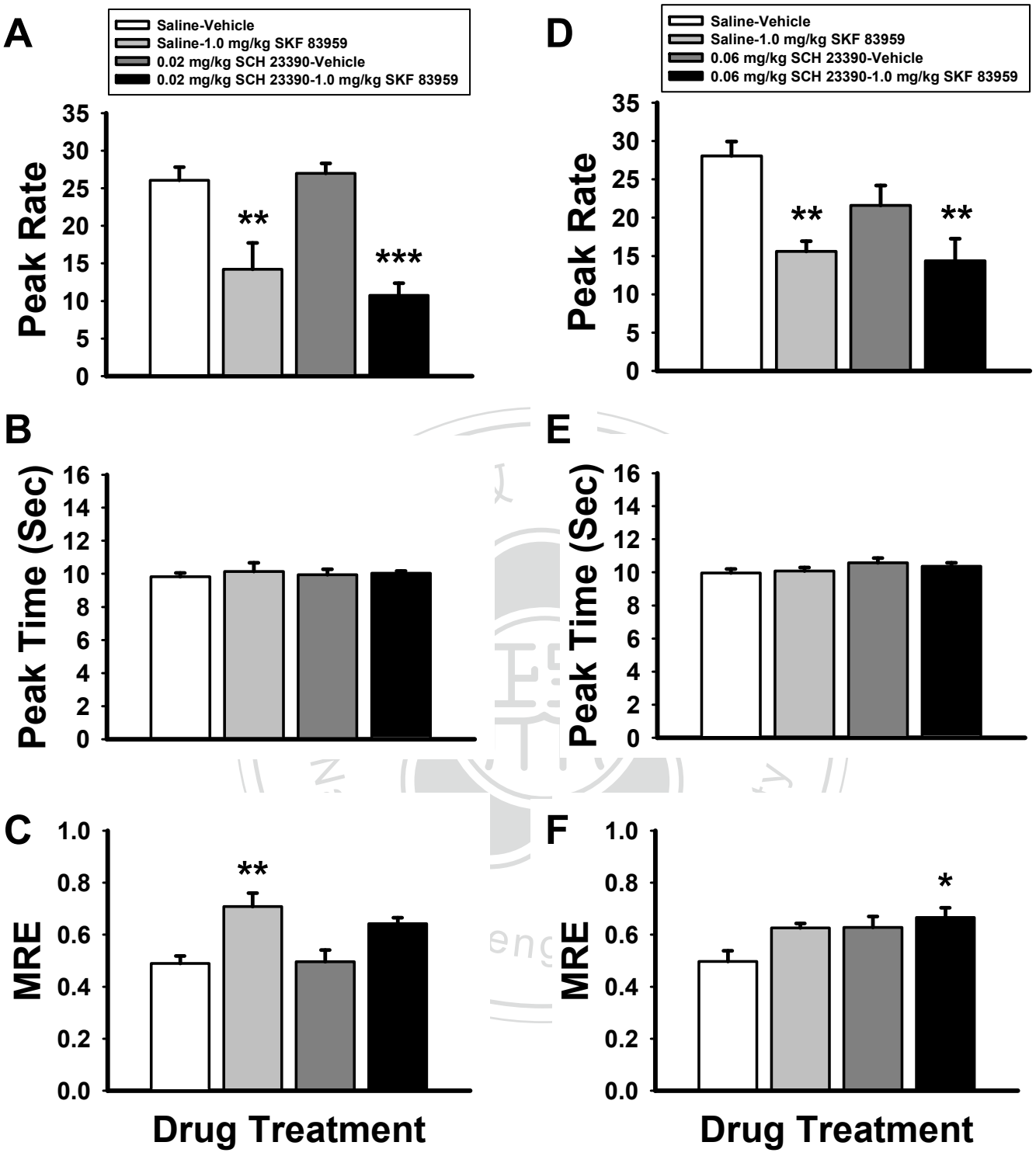

Figure 23. The DRL 10-s peak rate, peak time, and MRE after SCH 23390 pretreatment (n = 9): 0.02 mg/kg SCH 23390 (A, B, C) and 0.06 mg/kg SCH 23390 (D, E, F). Neither doses of SCH 23390 reversed the SKF 83959-induced decline in peak rates. The drug treatments did not affect the peak time. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ in comparison to respective vehicle. (Exp.4)

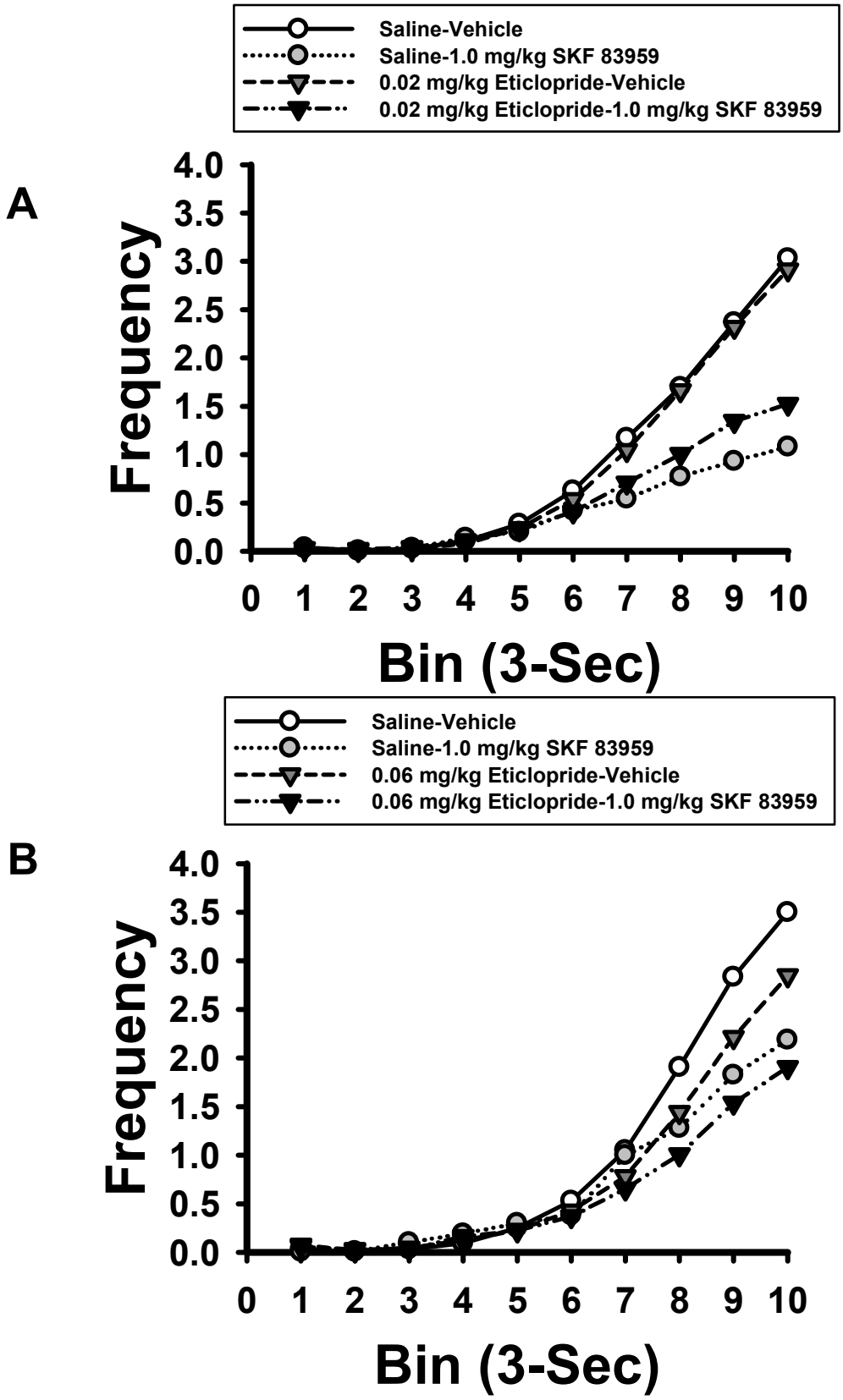

Figure 24. The FI 30-s IRT curve after eticlopride pretreatment in a within-subjects design (n = 9): 0.02 mg/kg eticlopride (A) and 0.06 mg/kg eticlopride (B) Low dose eticlopride appeared to partially reverse the SKF 83959-induced decline in response frequency on the FI 30-s schedule, while high dose eticlopride did not have such an effect. (Exp.4)

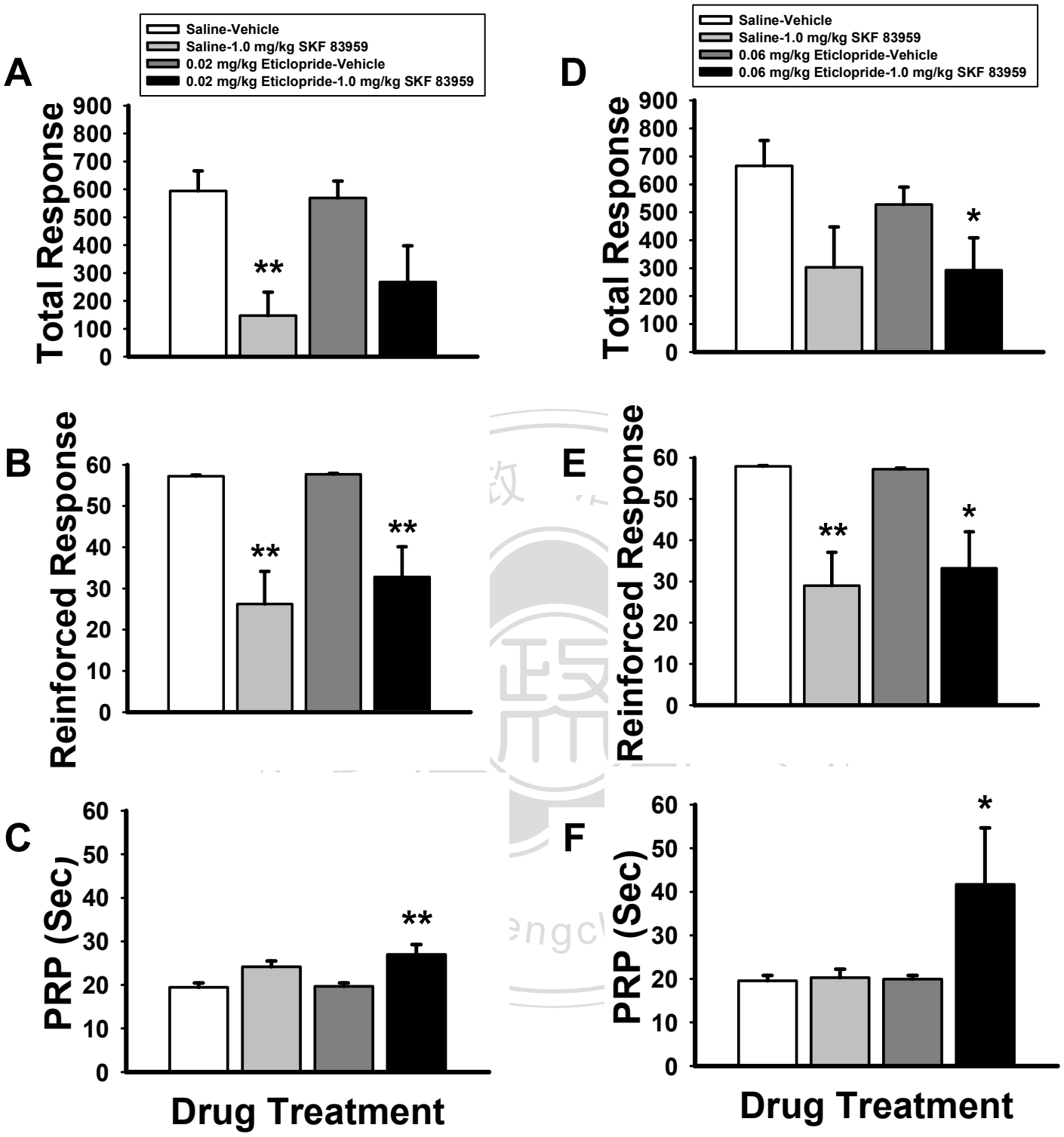

Figure 25. The FI 30-s indexes after eticlopride pretreatment (n = 9): 0.02 mg/kg eticlopride (A, B, C) and 0.06 mg/kg eticlopride (D, E, F). Low dose eticlopride had a partial reversal effect on the SKF 83959-induced decline in total response rate on the FI 30-s schedule. * $p < 0.05$; ** $p < 0.01$ relative to vehicle. (Exp.4)

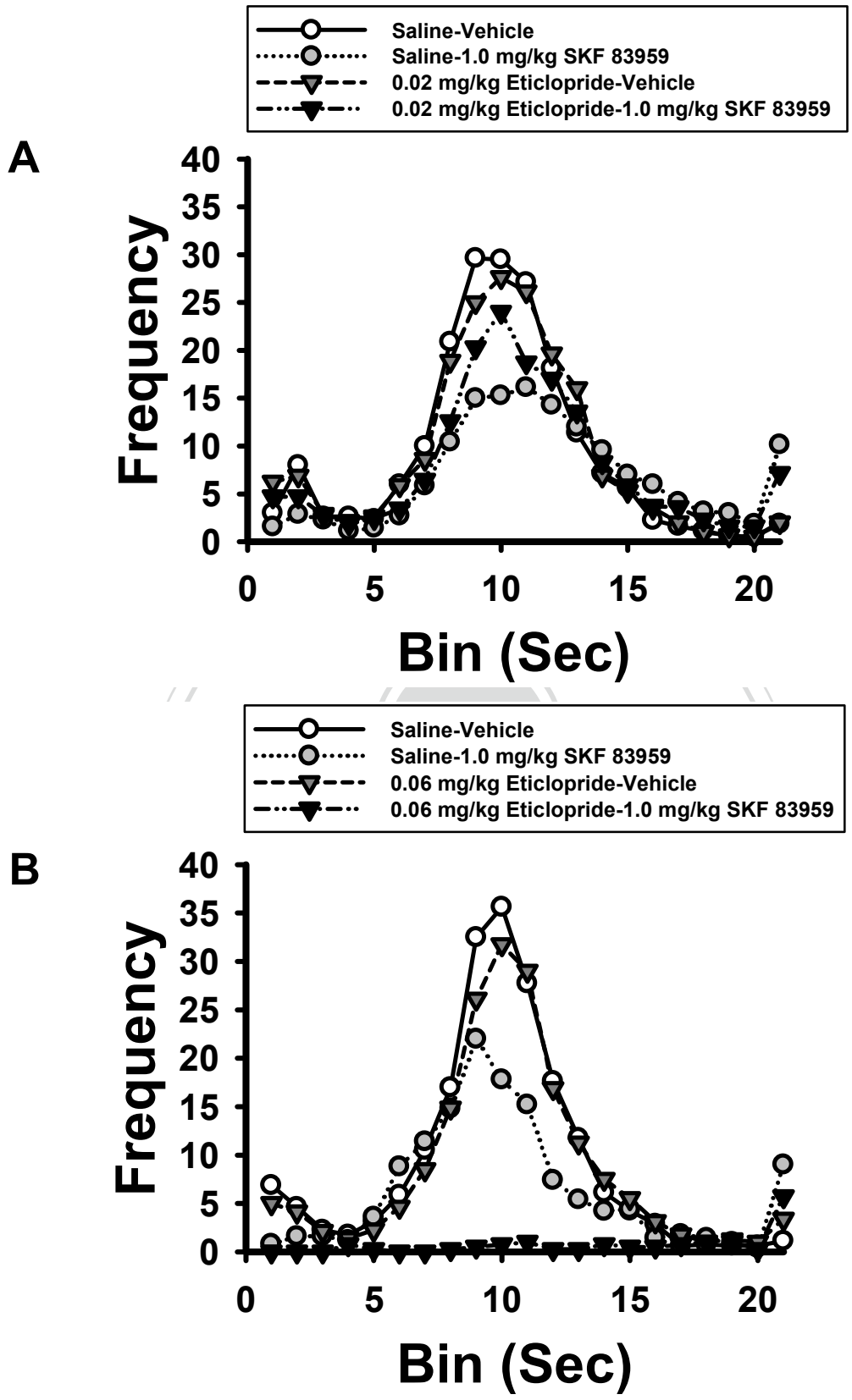

Figure 26. The DRL 10-s IRT curve after eticlopride pretreatment in a within-subjects design (n = 8): 0.02 mg/kg eticlopride (A) and 0.06 mg/kg eticlopride (B). Low dose eticlopride appeared to partially reverse the SKF 83959-induced decline in response frequency on the DRL 10-s schedule, while high dose eticlopride almost completely diminished the response frequency. (Exp.4)

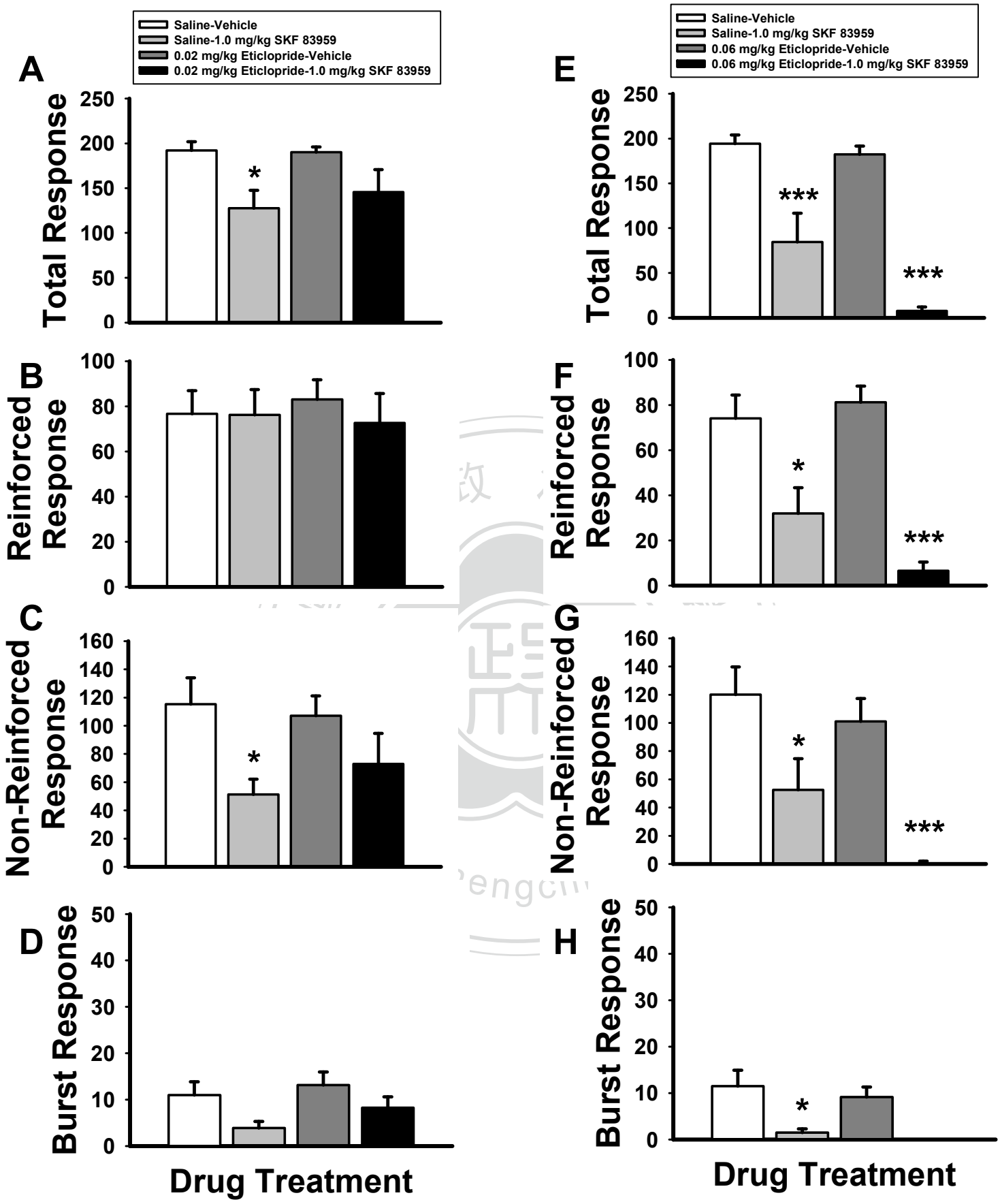

Figure 27. The DRL 10-s response-based indexes after eticlopride pretreatment (n = 8): 0.02 mg/kg eticlopride (A, B, C, D) and 0.06 mg/kg eticlopride (E, F, G, H). Low dose eticlopride had a partial reversal effect on the SKF 83959-induced declines in response rates on the DRL 10-s schedule. High dose eticlopride did not have such an effect. * $p < 0.05$; *** $p < 0.001$ relative to vehicle. (Exp.4)

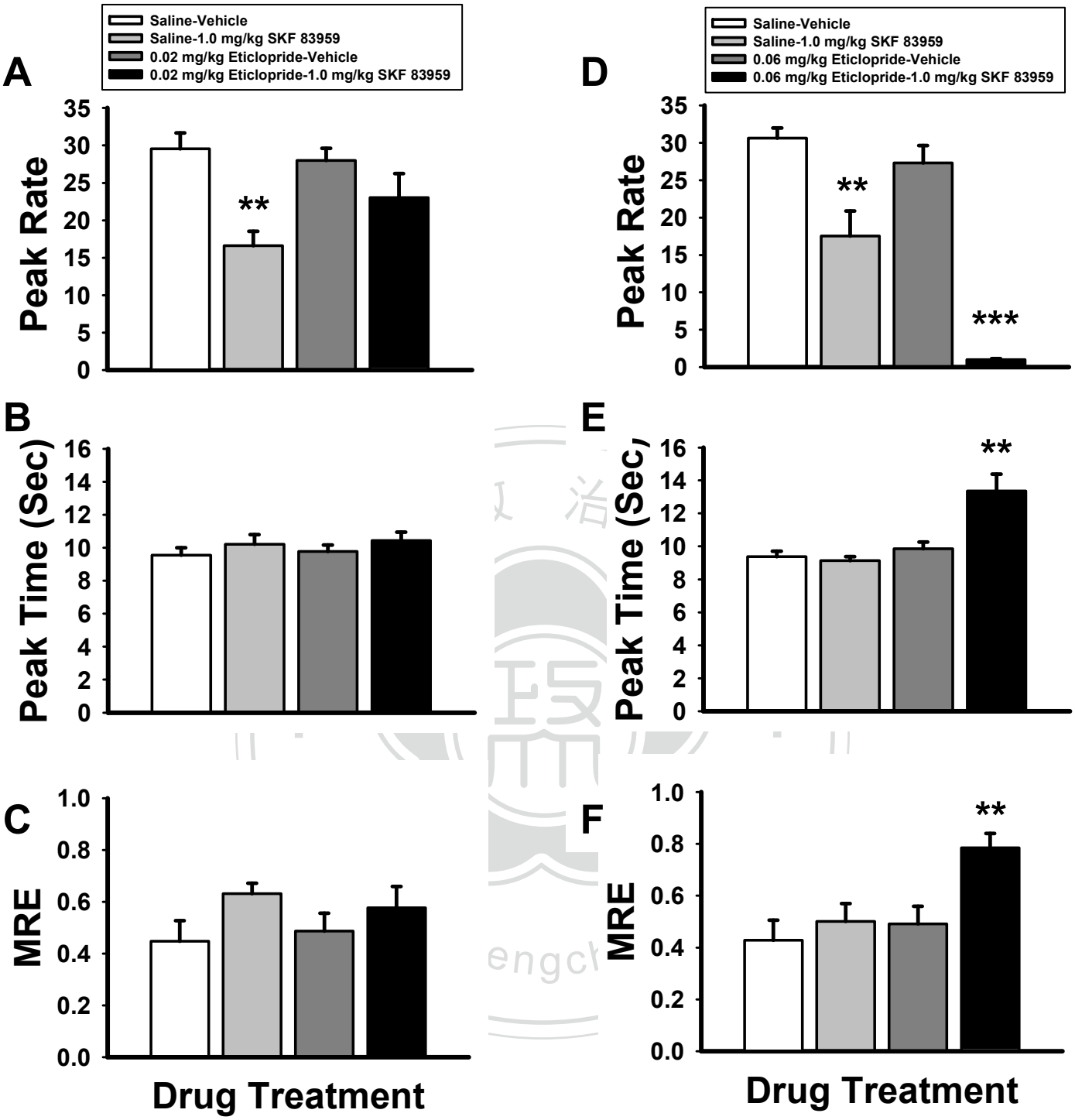

Figure 28. The DRL 10-s peak rate, peak time, and MRE after eticlopride pretreatment (n = 8): 0.02 mg/kg eticlopride (A, B, C) and 0.06 mg/kg eticlopride (D, E, F). Low dose eticlopride had a partial reversal effect on the SKF 83959-induced decline in peak rate. High dose eticlopride did not have such a reversal effect. ** $p < 0.01$; *** $p < 0.001$ in comparison to the respective vehicle. (Exp.4)

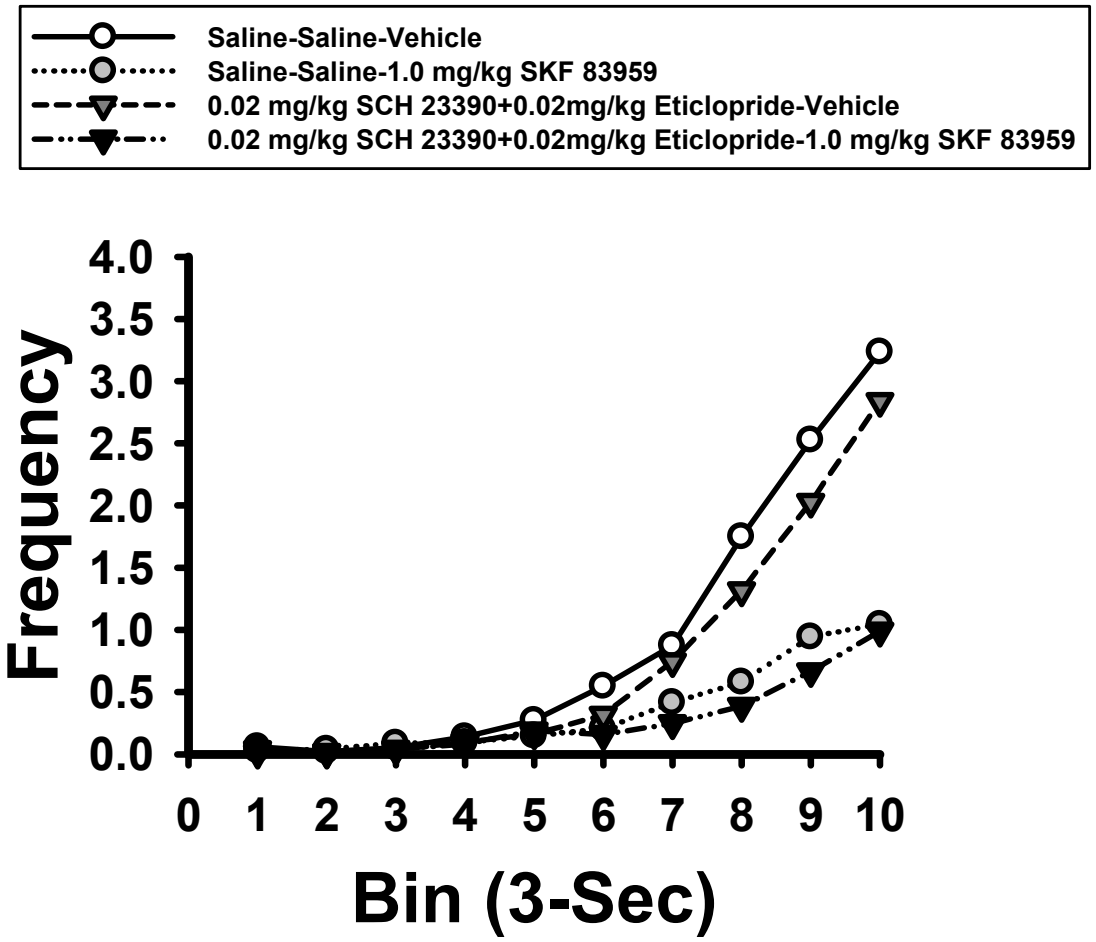

Figure 29. The FI 30-s IRT curve after the combined administration of 0.02 mg/kg SCH 23390 and 0.02 mg/kg eticlopride in a within-subjects design (n = 9). The co-administration of SCH 23390 and eticlopride did not reverse the SKF 83959-induced decline in response frequency on the FI 30-s schedule. (Exp.4)

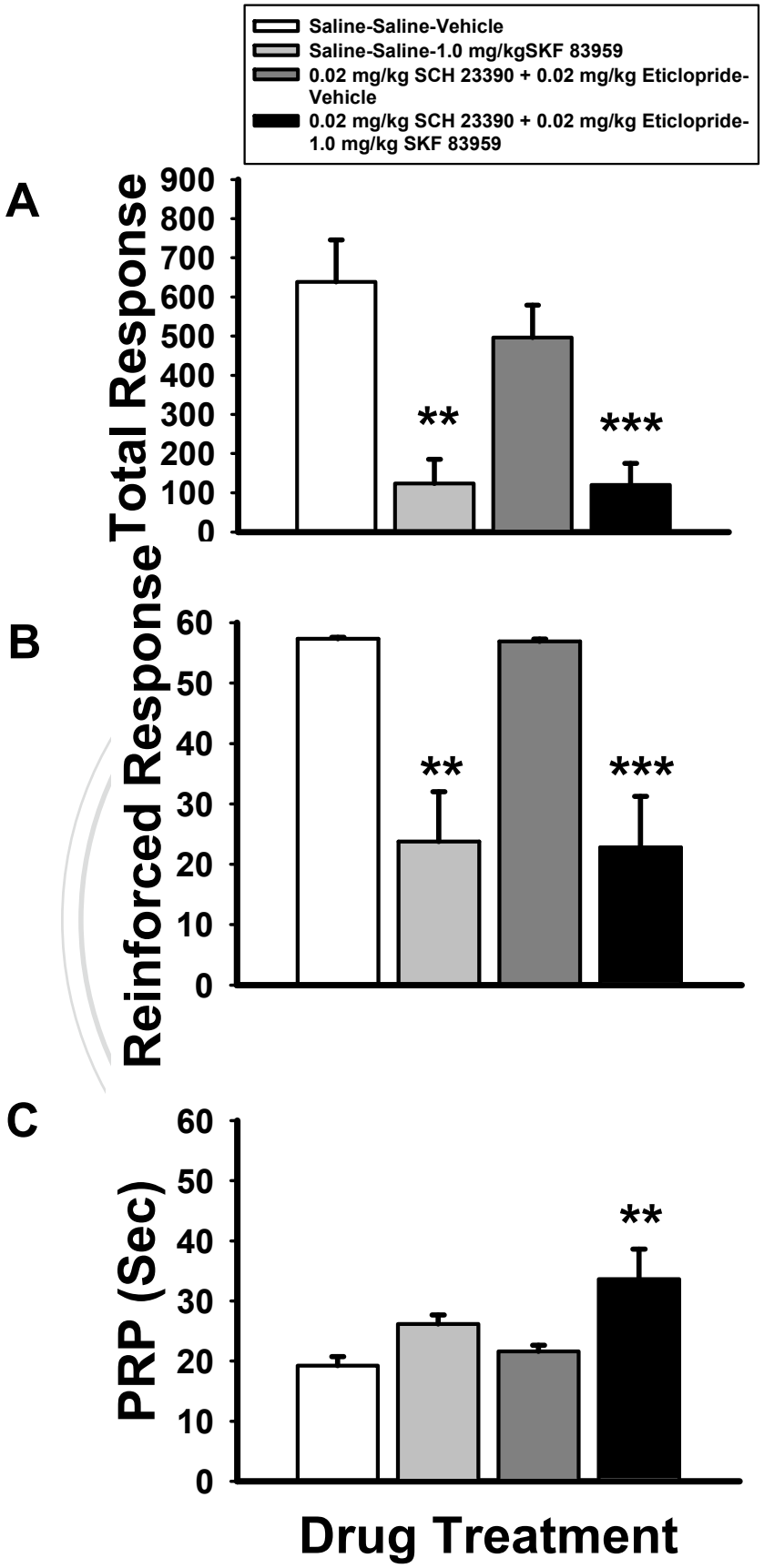

Figure 30. The FI 30-s indexes after the combined administration of 0.02 mg/kg SCH 23390 and 0.02 mg/kg eticlopride (n = 9): total response (A), reinforced response (B), and PRP (C). The co-administration of SCH 23390 and eticlopride did not reverse the effects of SKF 83959-on the FI 30-s indexes. ** $p <$ 0.01; *** $p < 0.001$ to the respective vehicle.(Exp.4)[126]

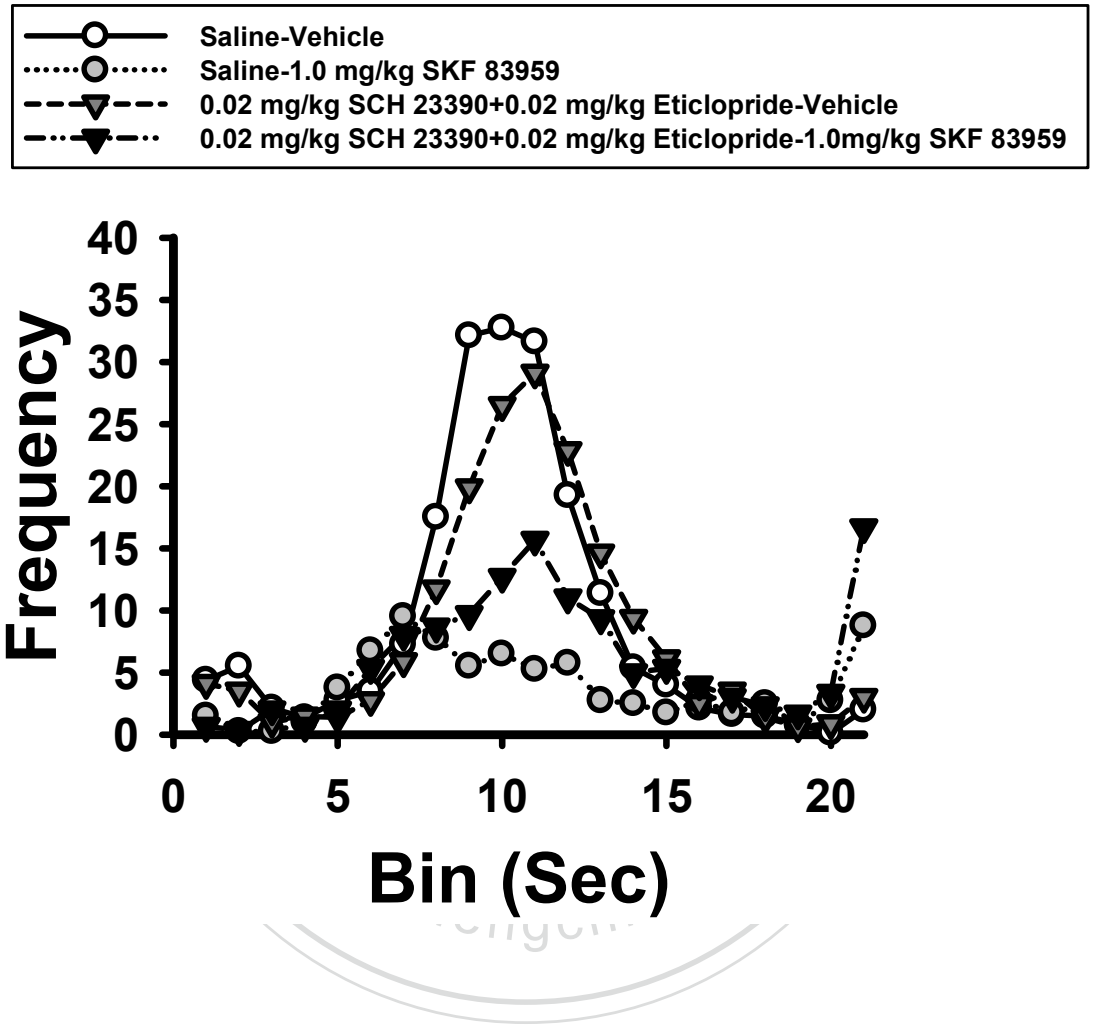

Figure 31. The DRL 10-s IRT curve after the combined administration of 0.02 mg/kg SCH 23390 and 0.02 mg/kg eticlopride in a within-subjects design (n = 8). (Exp.4)

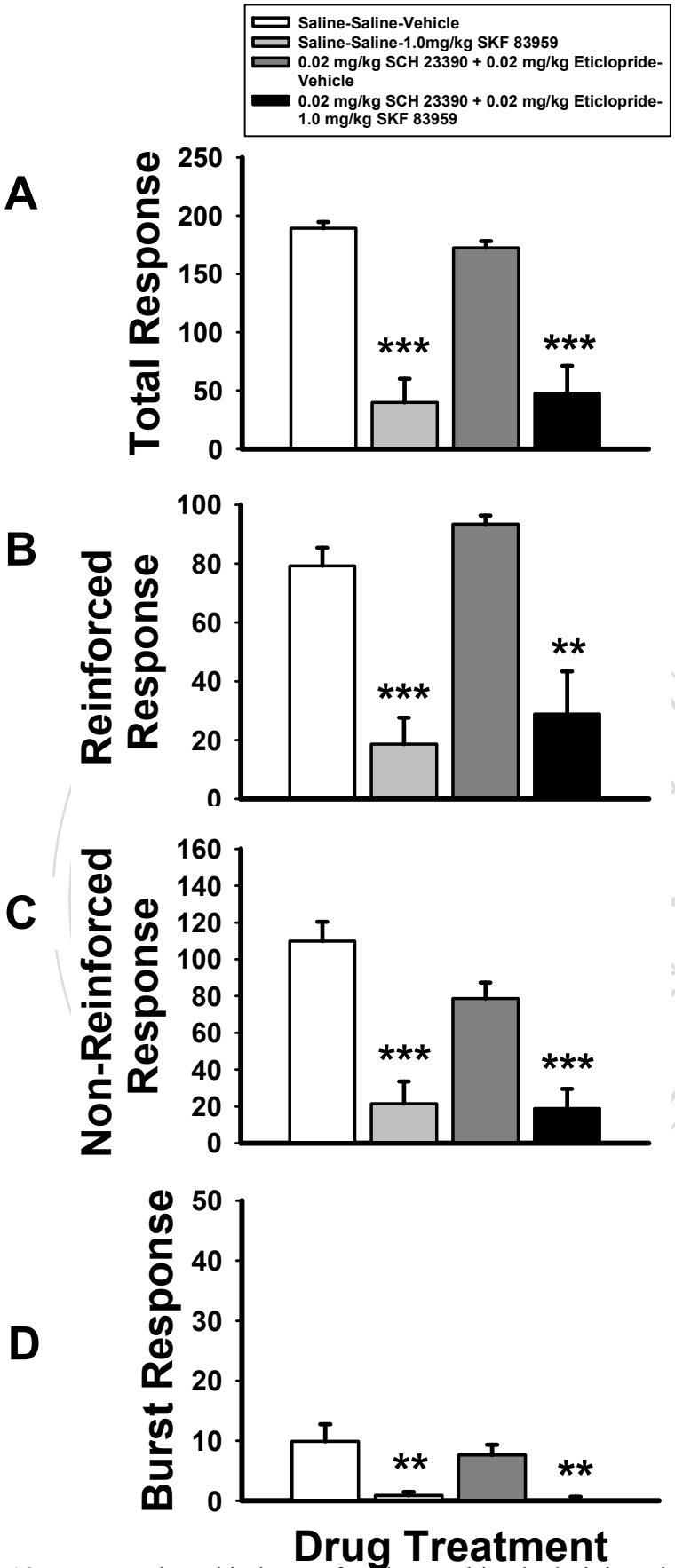

Figure 32. The DRL 10-s reponse-based indexes after the combined administration of 0.02 mg/kg SCH 23390 and 0.02 mg/kg eticlopride (n = 8). The co-administration of SCH 23390 and eticlopride did not reverse the SKF 83959-induced declines in response rates. ** $p < 0.01$; *** $p < 0.001$ in comparison to respective vehicle. (Exp.4)

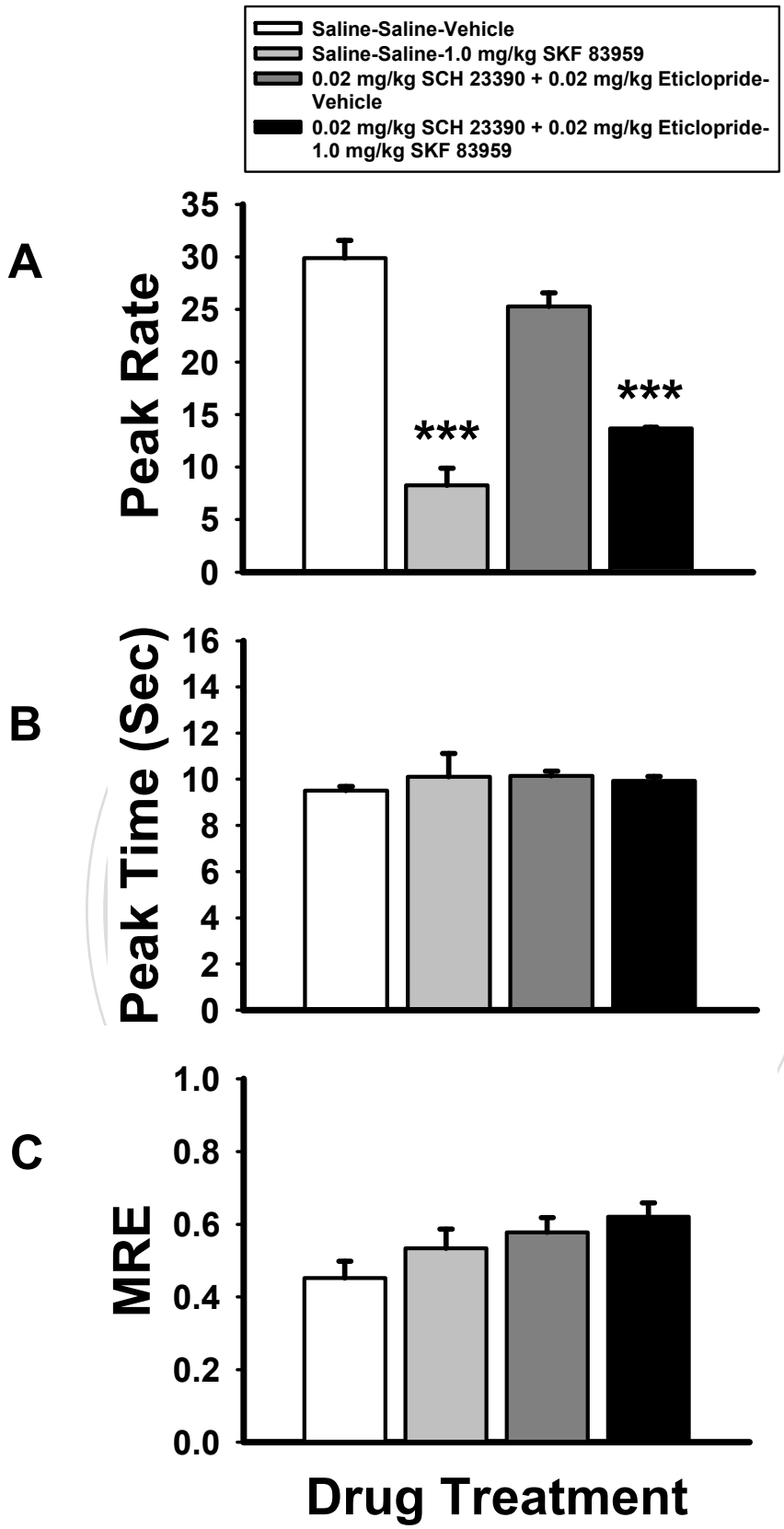

Figure 33. The DRL 10-s peak rate (A), peak time (B), and MRE (C) after the combined administration of 0.02 mg/kg SCH 23390 and 0.02 mg/kg eticlopride (n = 8). The co-administration of SCH 23390 and eticlopride did not reverse the SKF 83959-induced decline in peak rate on the DRL 10-s schedule. *** $p < 0.001$ in comparison to the respective vehicle. (Exp.4)

Appendix

A List of Abbreviated Words

Adenylyl cyclase (AC)

Calcium and calmodulin-dependent protein kinase II (CaMKII)

Cyclic adenosine monophosphate (cAMP)

Cyclic AMP response element binding protein (CREB)

Differential reinforcement of low rate (DRL)

Dopamine (DA)

Dopamine and cyclic AMP-regulated phosphoprotein, 32 KDa (DARPP-32)

Dopamine receptor (DAR)

Dorsal striatum (DS)

Extracellular signal regulated kinase (ERK)

Fixed interval (FI)

Fixed ratio (FR)

Guanosine-5'-triphosphate (GTP)

Human embryonic kidney cells (HEK cells)

Intracranial self-stimulation (ICSS)

Inter-response time (IRT)

Medial forebrain bundle (MFB)

Modified response efficiency (MRE)

Norepinephrine (NE)

Nucleus accumbens (NAc)

Phosphorylated CaMKII (pCaMKII)

Phosphorylated CREB (pCREB)

Phosphorylated ERK (pERK)

Phosphoinositide (PI)

Phospholipase C (PLC)

Prefrontal cortex (PFC)

Protein kinase A (PKA)

Post-reinforcement pause (PRP)

Total response (TR)