BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jan; 17 (1)

dependence. Stat Sci 1992; 7: 404—27.
3 McManns OB, Magleby KL. Kinetic time constants independent of previous single-channel activity suggest markov gating for a large conductance Ca-activated K channel. J Gen Physiol 1989; 94: 1037—69.
4 P-Clamp [computer program]. version 5.51. USA: Axon Instruments Inc, 1984.
5 Ye JH, Fan J, Chen ZH, Chen PX. Kinetic properties of a delayed rectifier potassium channels in PC12 cells. Acad J Sun Yat-Sen Univ Med Sci 1991; 12: 250—3.
6 SAS [computer program]. version 6.04. Cary NC: SAS Institute Inc, 1985.
7 Ball FG, Sansom MSP. Single-channel auto-correlation function: the effects of time interval omission. Biophys J 1988; 53: 819—32.
8 Hoshi T, Adrich RW. Gating kinetics of four classes of voltage-dependent K⁺ channels in pheochromocytoma cells. J Gen Physiol 1988; 91: 108—31.
9 Liebovitch LS, Koniarek JP. Ion channel kinetics, protein switching between conformational states is fractal in time. IEEE Eng Med Biol 1992; June: 53—6.

离子通道记忆的二状态随机模型[1]

方积乾，倪涛洋，傅承主，类 菁[2]，关永源[3]
（中山医科大学医学统计教研室，[2] 生理教研室，[3] 药理教研室，广州 510089，中国）

关键词 离子通道；统计学模型；记忆

目的：定量研究存在于通道中的记忆并提供有关统计方法以应用于生物医学研究。**方法**：利用随机过程分别建立短期和长期两类记忆的模型，坚持两状态而不是多状态，只是转移机制不同。**结果**：恒定转移强度的二状态马氏过程较好地拟合短期记忆的情形，处于一类随机环境中的二状态马氏过程较好地拟合长期记忆的情形；提出了参数估计的方法并以 PC12 细胞通道作为示例。**结论**：离子通道中的记忆可以用仅含两个状态的随机过程定量地建立模型。

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jan; 17 (1): 18—22

(—)-Stepholidine *vs* 12-chloroscoulerine enantiomers on firing activity of substantia nigral dopamine neurons[1]

ZHANG Xue-Xiang, JIN Guo-Zhang[2]
(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS stepholidine; 12-chloroscoulerine; electrophysiology; dopamine receptors; reserpine; substantia nigra; apomorphine; benzazepines

AIM: To compare the potencies between (—)-stepholidine ((—)-SPD) and 12-chloroscoulerine (CSL) enantiomers on firing of substantia nigra (SN) dopamine (DA) neurons. **METHODS**: Extracellular single unit recordings in paralyzed rats. **RESULTS**: In rats, (—)-SPD, (—)-, (±)-, and (+)-CSL attenuated iv apomorphine (Apo, 10 $\mu g \cdot kg^{-1}$)-induced inhibition on firing of DA cell, and their ED_{50} values were 15.1 (11.9 — 19.4), 7.8 (7.0 — 8.7), 12.6 (2.0 — 17.9) $\mu g \cdot kg^{-1}$, and 2.9 (2.6—3.3) $mg \cdot kg^{-1}$, respectively. Thus, (—)-CSL was 1 time more potent than (—)-SPD and 371 times more potent than (+)-CSL on D_2 receptors. In reserpinized rats, (—)-SPD, (—)-, (±)-, and (+)-CSL blocked the inhibition caused by iv 4 $mg \cdot kg^{-1}$ SKF-38393, with ED_{50} values of 0.53 (0.51 — 0.55), 0.51 (0.43—0.60), 1.2 (0.7—2.0), and 5.9 (4.9 — 7.1) $mg \cdot kg^{-1}$, respectively. The potency of (—)-CSL was similar to that of (—)-SPD on D_1 receptors and 11 times higher than that of (+)-CSL. **CONCLUSION**: CSL enantiomers are D_1/D_2 mixed antagonists as (—)-SPD. (—)-CSL is the most, while (+)-CSL is the least, potent one among them.

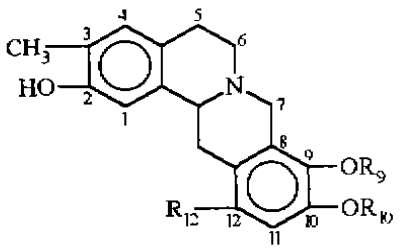
Dopamine (DA) receptors have been divided into D_1 and D_2 subtypes[1]. Autoreceptors, a special subpopulation of D_2 receptors, concentrate on presynaptic terminals and soma-dendrites of DA neurons in substantia nigra pars compacta

[1] Project supported by the National Natural Science Foundation of China, № 39320001, 39130091.
[2] Correspondence to Prof JIN Guo-Zhang.
Received 1995-03-24 Accepted 1995-06-06

(SNC). Besides, postsynaptic cells in striatum contain both D_1 and D_2. More D_1 receptors locate in substantia nigra pars reticulata (SNR) which is the output nucleus of striatum[2]. DA agonists act preferentially at somatodendritic autoreceptors to inhibit DA cell firing[3], while DA antagonists attenuate the inhibition through D_2 subtype[4]. Thus firing of SNC DA cell in normal rats is used to evaluate the effects of selective D_2 DAergic drugs.

On the other hand, in nonreserpinized rats, D_1 agonists, ie SKF-38393 ((±)-1-phenyl-2, 3, 4, 5-tetrahydro-(1*H*)-3-benzazepine-7, 8-diol), induce no effect on firing of DA cells. In contrast, in rats pretreated with reserpine for 6 d, SKF-38393 commits a marked inhibition which is readily blocked by D_1 but not D_2 antagonists[5,6]. Reserpine pretreatment seems to render D_1 receptors "supersensitive" to D_1 agonist[7]. Therefore, reserpinized rats were used to assess the potencies of D_1 antagonists.

(−)-Stepholidine (SPD), a novel DA antagonist[8,9], is difficult to be industrially synthesized. 12-chloroscoulerine (CSL) enantiomers, same as SPD, belong to tetrahydroprotoberberines (THPB). Except (±)-CSL, (+)- and (−)-CSL were synthesized for the first time in the world by Zhou (unpublished data). The characteristics on DA receptors of these new compounds have not been systematically studied with electrophysiological methods. Hence, we conducted the present study to assess the potencies of CSL enantiomers on D_1 and D_2 receptors with comparison of those of (−)-SPD.



R_9	R_{10}	R_{12}	
CH_3	H	H	Stepholidine
H	CH_3	Cl	Chloroscoulerine

Tetrahydroprotoberberines

MATERIALS AND METHODS

Rats and pretreatment Sprague-Dawley rats (clean, ♂, $n=60$, $220\pm s$ 27 g, Shanghai Experimental Animal Center, Shanghai. Certification № 005 conferred by Animal Management Committee, Chinese Academy of Sciences.) were used. The rats were randomly divided into 2 groups. One group ($n=28$) was kept naive to test the potencies of compounds on D_2 subtype. The others ($n=32$) were pretreated with sc reserpine (1 mg·kg^{-1}·d^{-1}) for 6 consecutive days and recordings were made 3 − 6 h after the last injection. The reserpinized rats were used to elucidate the effects of drugs on D_1 subtype.

Single unit recording technique Extracellular single unit recordings were performed in paralyzed rats[10]. A small burr hole, 2.2 mm lateral to midline and 3.2 mm anterior to the lambdoid suture[11], was drilled through the skull for recording in SNC. Electric signals picked up by the glass microelectrode (filled with NaCl 2 mol·L^{-1} containing 1 % pontamine sky blue, 3 − 9 MΩ measured *in vitro*) were amplified and led into a window discriminator. Firing rate was counted by a computer. Body temperature was maintained at 36 − 38 ℃ with an electric heating pad.

The identification of neurons as putative SNC DA cells was based on the well-established indices[12]. For each DA receptor subtype studied, after a 5-min period of baseline activity was recorded, the first injection of drugs started. In the D_2 subtype experiments, 2 min after apomorphine (Apo) was iv given, (−)-SPD and CSL enantiomers were iv at 2 min interval so that each dose doubled the previous one. In reserpinized rats, a single dose of SKF-38393 was iv given 10 min before iv (−)-SPD or CSL enantiomers. Only one DA cell was monitored per rat.

Drugs Apomorphine-HCl (Shenyang Pharmaceutical Co, China); SKF-38393-HCl (Research Biochemicals International, USA); gallamine triethiodide (Sigma, USA); lidocaine-HCl (Shanghai Haipu Pharmaceutical Co, China); pontamine sky blue (Merck, USA); (−)-SPD ($[\alpha]_D$ − 440°, pyridine), (−)-CSL ($[\alpha]_D$ − 223.19°, $CHCl_3$), (+)-CSL ($[\alpha]_D$ + 222.53°, $CHCl_3$) and (±)-CSL enantiomers were prepared by Shanghai Institute of Materia Medica and dissolved in a slightly acidic solution at pH 4.0 − 4.5 (in H_3PO_4 0.2 % adjusted by NaOH 0.2 mol·L^{-1}).

Statistics Data were expressed as $\bar{x}\pm s$. The ED_{50} and its 95 % confidential limits for the reversal effects of each compound was estimated by logit method.

RESULTS

Reversal of Apo-induced inhibition on firing activity of nigral DA neurons for D_2 receptors In nonreserpinized rats, D_1/D_2 agonist Apo at a

low dose of 10 $\mu g \cdot kg^{-1}$, iv, profoundly depressed the firing rate of nigral DA neurons by more than 90 % (Fig 1) and the inhibition persisted for at least 1 h.

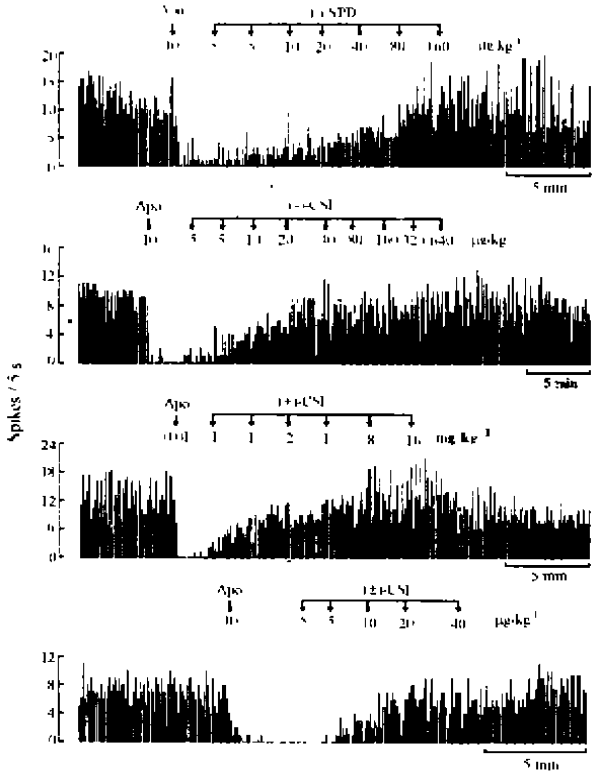


Fig 1. Inhibition of firing of SNC DA cells induced by Apo was attenuated by (−)-SPD and CSL enantiomers.

Two minutes after the inhibition, (−)-SPD was iv and the inhibition of Apo was abolished completely. Attenuation reached 130 ± 40 % when the cumulative dose was 320 $\mu g \cdot kg^{-1}$. (±)-CSL had the similar effect in a dose-dependent way. However, (−)-CSL dose-dependently antagonized the inhibition until the attenuation reached <90 % at the cumulative dose of 80 $\mu g \cdot kg^{-1}$. Afterwards dispensation of (−)-CSL did not block the inhibition any more and the attenuation curve maintained at the plateau even at the dose 1280 $\mu g \cdot kg^{-1}$ (Fig 2).

ED_{50} values of (−)-SPD, (−)-CSL, and (±)-CSL were 15.1 (11.9−19.4, $n=6$), 7.8 (7.0−8.7, $n=6$), and 12.6 (7.0−17.9, $n=6$) $\mu g \cdot kg^{-1}$, respectively. (+)-CSL had little effect to attenuate the Apo-induced inhibition until the dosage was on the order of $mg \cdot kg^{-1}$. The attenuation rate was 138±38 % following (+)-CSL 32 $mg \cdot kg^{-1}$ treatment (Fig 1,2). Its ED_{50} was 2.9 (2.7−3.3, $n=10$) $mg \cdot kg^{-1}$, which was approximately 371 times higher than that of (−)-CSL.

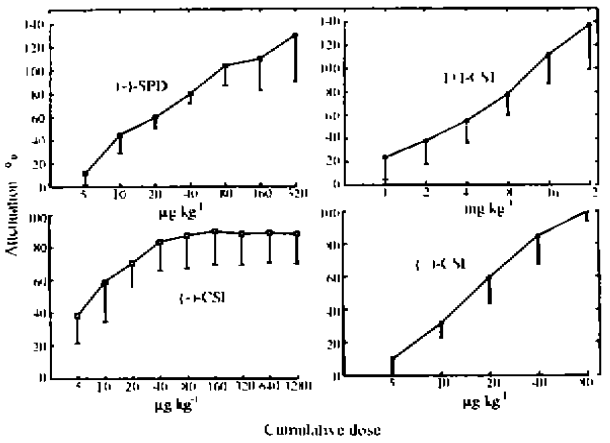


Fig 2. Attenuation of Apo (10 $\mu g \cdot kg^{-1}$)-induced inhibition of firing of nigral DA cells. ($n=6-10$).

Potencies on SKF-38393-induced inhibition in reserpinized rats for D_1 receptors A single bolus iv of selective D_1 receptor agonist SKF-38393 4 $mg \cdot kg^{-1}$ inhibited the spontaneous firing of DA neurons in rats pretreated with reserpine (Fig 3).

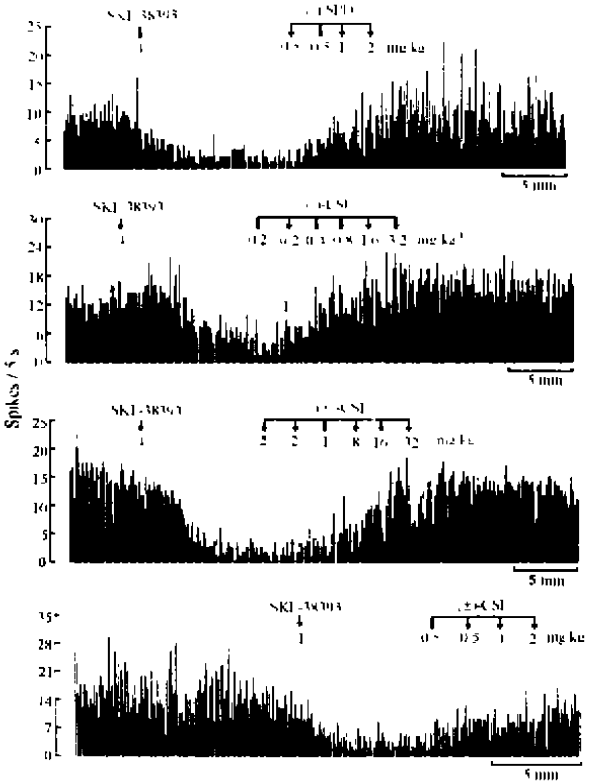


Fig 3. Attenuation of the inhibition of firing of SNC DA cells caused by SKF-38393 in reserpinized rats by (−)-SPD or CSL enantiomers.

This inhibitory effect reached the peak at 10

min after iv and was readily attenuated by iv (−)-SPD (0.5−4 mg·kg^{-1}), (−)-CSL (0.2−6.4 mg·kg^{-1}), (±)-CSL (0.5−4 mg·kg^{-1}), or (+)-12-CSL (2−64 mg·kg^{-1}). All these compounds diminished the inhibition thoroughly (Fig 3,4). ED_{50} values of (−)-SPD, (−)-, (±)-, and (+)-CSL were 0.53 (0.51−0.55, n=9), 0.51 (0.43−0.60, n=8), 1.2 (0.7−2.0, n=7), and 5.9 (4.9−7.1, n=8) mg·kg^{-1}, respectively.

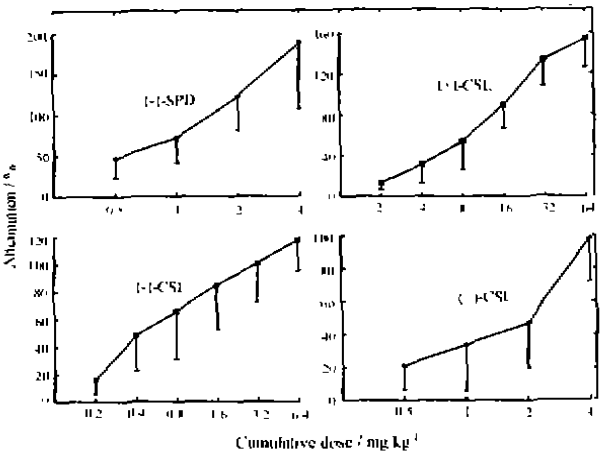


Fig 4. Attenuation of SKF-38393-caused suppression in reserpinized rats by (−)-SPD and CSL enantiomers. Rats were pretreated with SKF-38393 4 mg·kg^{-1} 10 min prior to the first injection of (−)-SPD or CSL enantiomers (n=7−9).

DISCUSSION

This study demonstrated that (−)-SPD, (−)-, and (±)-CSL were D_2 antagonists. (+)-CSL, however, is very weak one. This result is consistent with our previous results from radioligand binding assay that the affinity of (+)-CSL was very weak to D_2 subtype (unpublished data).

Under normal conditions, D_1 receptors express enabling effects with D_2 rather than its independent roles. It is difficult to get ideal samples to assess D_1 DAergic drugs. For example, in normal rats, selective D_1 agonist SKF-38393 did not show any effect on the firing of SNC DA cells. Nevertheless, after reserpinization, SKF-38393 profoundly inhibited the firing[6,7]. In contrast, selective D_2 agonists inhibited the firing in reserpinized as well as in nonreserpinized rats and the potencies were very similar in both models[11]. These inhibitory effects of selective D_1 or D_2 agonists in the reserpinized rats could only be attenuated by their respective antagonists. Therefore, the reserpinized rat is used as a tentative model to assay the D_1 action of drugs such as (−)-SPD and CSL enantiomers, though the mechanisms underlying the independent expression of D_1 is not clear yet. Preliminary data in our laboratory demonstrated that the integrity of SNC-striatum-SNR circuits were indispensable for the inhibition of SKF-38393. The conclusion was reasonable because in reserpinized rats, SKF-38393 did not alter the firing when it was locally iontophoresed into SNR. Furthermore, interruption of striatum-SNR projection abolished the effect of SKF-38393 (to be published).

It should be stated that it is better to do experiments on vehicle control of reserpine and CSL enantiomers. Early work on the reserpinized model showed that chronic administration of normal saline induced no effects on the spontaneous locomotor activity of rats[14]. Our previous work has also demonstrated that vehicle of (−)-SPD did not affect the inhibition induced by Apo[15]. Nevertheless, the present work was done under different conditions and the vehicles were somewhat different.

Conclusively, CSL enantiomers were D_1/D_2 mixed antagonists as (-)-SPD. The similarities of characteristics may be due to the similar structure of CSL to (−)-SPD.

REFERENCES

1 Kebabian JW, Calne DB. Multiple receptors for dopamine. Nature 1979; **277**: 93−6.

2 Filloux FM, Wamsley JK, Dawson TM. Presynaptic and postsynaptic D_1 dopamine receptors in the nigrostriatal system of the rat brain: a quantitative autoradiographic study using the selective D_1 antagonist [^{3}H]SCH 23390. Brain Res 1987; **408**: 205−9.

3 Wang RY, White FJ, Voigt MM. Effects of dopamine agonists on midbrain dopamine cell activity. In: Usdin E, Carlsson A, Dahlström A, Engel J, editors. Catecholamines part B: neuropharmacology and central nervous system-theoretical aspects. New York: Alan R Liss, 1984; 359−67.

4 Chiodo LA, Bunney BS. Effects of dopamine antagonists on midbrain dopamine cell activity. In: Usdin E, Carlsson A, Dahlström A, Engel J, editors. Catecholamines part B: neuropharmacology and central nervous system-theoretical aspects. New York: Alan R Liss, 1984; 369−91.

5 Sun BC, Jin GZ. Characteristics of (-)-stepholidine on the firing activity of substantia nigral dopamine neurons after repeated reserpine treatment. Biol Signals 1992; **1**: 331−8.

6 Huang KX, Walters JR. D_1 receptor stimulation inhibits dopamine cell activity after reserpine treatment but not after chronic SCH 23390: an effect blocked by N-methyl-D-aspartate antagonists.
J Pharmacol Exp Ther 1992; **260**: 409－416.

7 Missale C, Nisoli E, Liberini P, Rizzonelli P, Memo M, Buonamici M, *et al*. Repeated reserpine administration up-regulates the transduction mechanisms of D_1 receptors without changing the density of [^{3}H]SCH 23390 binding.
Brain Res 1989; **483**: 117－22.

8 Xu SX, Yu LP, Han Y, Chen Y, Jin GZ. Effects of tetrahydroprotoberberines on dopamine receptor subtypes in brain.
Acta Pharmacol Sin 1989; **10**: 104－10.

9 Huang KX, Sun BC, Jin GZ. (-)-Stepholidine: a dopamine receptor antagonist shows agonistic effect on rotational behavior in 6-hydroxydopamine-lesioned rats.
Acta Pharmacol Sin 1992; **13**: 17－22.

10 Bunney BS, Walters JR, Roth RH, Aghajanian GK. Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity.
J Pharmacol Exp Ther 1973; **185**: 560－71.

11 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Sydney: Academic Press. 1982.

12 Skirboll LR, Grace AA, Bunney BS. Dopamine auto- and postsynaptic receptors: electrophysiological evidence for differential sensitivity to dopamine agonists.
Science 1979; **206**: 80－2.

13 Sun BC, Huang KX, Jin GZ. Repeated reserpine treatment alters firing pattern and responses of substantia nigral dopamine neurons. Eur J Pharmacol 1993; **231**: 331－8.

14 Pirch JH, Rech RH. Behavioral recovery in rats during chronic reserpine treatment.
Psychopharmacologia 1968; **12**: 115－22.

15 Sun BC, Huang KX, Jin GZ. Comparison of effects of tetrahydropalmatine enantiomers on firing activity of dopamine neurons in substantia nigra pars compacta.
Acta Pharmacol Sin 1992; **13**: 292－7.

左旋千金藤立定和12-氯斯阔任旋光异构体对黑质多巴胺神经元放电活动的比较

张雪翔，金国章
（中国科学院上海药物研究所．上海 200031，中国）

关键词 千金藤立定；12-氯斯阔任；电生理学；多巴胺受体；利血平；黑质；阿朴吗啡；苯二氮䓬类

目的：比较左旋千金藤立定（(－)-stepholidine，(－)-SPD）和12-氯斯阔任（12-chloroscoulerine，CSL）对黑质（substantia nigra，SN）多巴胺（DA）神经元放电的影响．**方法**：麻痹大鼠上的胞外单单位记录．**结果**：在大鼠，(－)-SPD，(－)-，(±)-和(＋)-CSL 减弱 iv 10 $\mu g \cdot kg^{-1}$ 阿朴吗啡引起的放电抑制．ED_{50} 值分别为 15.1（11.9－19.4），7.8（7.0－8.7），12.6（2.0－17.9）$\mu g \cdot kg^{-1}$ 和 2.9（2.6－3.3）$mg \cdot kg^{-1}$．(－)-CSL 比(－)-SPD 强1倍，比(＋)-CSL 强371倍．在利血平化大鼠，(－)-SPD，(－)-，(±)-，和(＋)-CSL 减弱 4 $mg \cdot kg^{-1}$ SKF-38393 引起的放电抑制．ED_{50} 值为 0.53（0.51－0.55），0.51（0.43－0.60），1.2（0.7－2.0）和 5.9（4.9－7.1）$mg \cdot kg^{-1}$．(－)-CSL 的强度与(－)-SPD 相似，比(＋)-CSL 强11倍．**结论**：(－)-SPD 和 CSL 是 D_1/D_2 混合性阻滞剂．(－)-CSL 最强，(＋)-CSL 最弱．

《中国疼痛医学杂志》创刊

由中华医学会疼痛学会和北京医科大学神经科学研究中心主办的《中国疼痛医学杂志》(Chinese Journal of Pain Medicine)创刊号于1995年9月正式出版．该刊为季刊，刊号 ISSN 1006-9852，CN11-3741R，每期64页．刊登与疼痛和镇痛有关的基础研究论文和临床论文．韩济生教授任主编．投稿或订阅的联系人：北京100083 北京医科大学神经科学研究中心 康妹娟．