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Effects of the acute administration of delta-opioid receptor ligands on the excitability of rat hippocampal glutamate and brainstem monoamine neurons *in vivo*

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Abstract. It was previously reported that the delta opioid receptor (DOR) agonist SNC80 and antagonist naltrindole modulate the excitability of hippocampal glutamate neurons in primary cultures. The present study aimed to investigate the acute effects of these ligands on the firing activity of hippocampal cornu ammonis 1/3 (CA1/3) glutamate, dorsal raphe nucleus (DRN) serotonin (5-HT), locus coeruleus (LC) noradrenaline, and ventral tegmental area (VTA) dopamine neurons in *in vivo* conditions. Adult Wistar male rats were used. SNC80 and naltrindole were administered intravenously. Neuronal firing activity was assessed using extracellular single-unit electrophysiology. SNC80, administered first at 1–3 mg/kg, dose-dependently inhibited CA1/3 glutamate, DRN 5-HT, and VTA dopamine neurons. Naltrindole, administered at 1–3 mg/kg after SNC80, did not have any additional effect. Naltrindole, administered first at 1–3 mg/kg, stimulated DRN 5-HT neurons in a dose-dependent manner; this stimulation was dose-dependently reversed by 1–3 mg/kg of SNC80. SNC80 and naltrindole inhibited LC noradrenaline neurons when only they were co-administered at 3 mg/kg, and only when SNC80 was administered first. In conclusion, DOR ligands alter the firing activity of hippocampal glutamate and brainstem monoamine neurons in *in vivo* conditions. The psychoactive effects of DOR ligands, reported in previous studies, might be explained, at least in part, by their ability to modulate the firing activity of hippocampal glutamate and brainstem monoamine neurons.

Key words: Delta-opioid receptor — Hippocampus — Dorsal raphe nucleus — Ventral tegmental area — Locus coeruleus

Introduction

Contemporary antidepressant drugs primarily act on central monoamine pathways, namely, on serotonin (5-HT), noradrenaline, and dopamine systems. Despite the certain progress with their safety and selectivity, the clinical efficiency of the currently existing remains limited. It is possible that other than monoaminergic targets should be elaborated for a proper therapeutic outcome (Dremencov et al. 2021). Opioid system is one of these possible targets (Varastehmoradi et al. 2020; Bodnar 2022). Several lines of evidence support this hypothesis. Thus, chronic treatment with opioids increased the risk of depression (Scherrer et al. 2015). On the other hand, an opioid buprenorphine showed effectiveness in the treatment of depression (Falcon et al. 2016) and post-traumatic stress disorder (PTSD) (Seal et al. 2016; Madison and Eitan 2020). Consistently, the antagonist of opioid receptors (OR) naltrexone was reported to have a depressogenic effect (Price et al. 2016).

Out of ORs, delta ORs (DORs) are of special interest, since they are responsible for the anxiolytic and antidepressant-like effects of opioids, but their involvement in the opioid addiction might be minor (Chu Sin Chung and Kieffer 2013). Anxiolytic (Saitoh et al. 2004, 2005) and antidepressant-like (Jutkiewicz et al. 2003, 2005a; Saitoh et al. 2004) effects were reported

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for (+)-4-(alpha-R)-alpha-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl-*N*,*N*-diethyl-benzamide (SNC80), an agonist of the DORs (Calderon et al. 1994).

We had recently reported that SNC80 increased the spontaneous firing activity of hippocampal neurons in primary culture. Since the selective DOR antagonist naltrindole had an opposite effect, and since pre-administration blocked the stimulatory effect of SNC80 on the spontaneous activity of hippocampal neurons, we suggested that the observed effect of SNC80 is indeed DOR-mediated (Moravcikova et al. 2021). The hippocampal neurons investigated by Moravcikova and colleagues were presumably pyramidal glutamatergic ones, as it was assumed from their morphology and characteristic ion currents across the membrane. Since hippocampal glutamate transmission is involved in antidepressant drugs response (Hlavacova et al. 2018; Lee et al. 2022), it is possible that anxiolytic and antidepressant-like effects of SNC80, observed in previous studies, are explained, at least in part, *via* the ability of this ligand to modulate the firing activity of hippocampal neurons. To test this hypothesis, the effect of SNC80, as well as of the selective DOR antagonist naltrindole, on the excitability of hippocampal glutamate neurons, should be tested in *in vivo* conditions.

Hippocampal functions, related to depression and antidepressant drug response, are linked with the interactions between hippocampal glutamate and brainstem monoamines (serotonin or 5-HT, noradrenaline, and dopamine). The axons of monoamine-secreting neurons densely innervate hippocampus and monoamines modulate the activity of hippocampal neurons. Certain antidepressant drugs alter monoaminergic modulation of the activity of hippocampal pyramidal neurons (Mongeau et al. 1997; Pavlovicova et al. 2015). It is thus possible that the modulation of hippocampal neurons by DOR ligands is mediated by monoamines.

DOR ligands may modulate the excitability of 5-HT neurons of the dorsal raphe nucleus (DRN), noradrenaline neurons of the locus coeruleus (LC), and dopamine neurons of the ventral tegmental area (VTA). Three lines of evidence support this hypothesis. Firstly, DRN and VTA neurons express enkephalins (Henry et al. 2017), and DRN, LC, and VTA are densely innervated by the axons of the β-endorphin-secreting neurons of the arcuate nucleus of the hypothalamus (Veening et al. 2012), suggesting that endogenous opioid and monoamine systems of the brain are interacting on the functional level. Since DORs are expressed in the DRN and VTA (Erbs et al. 2015) this type of ORs might play a role in opioid-monoamine interactions.

Secondly, it was shown that at least some behavioral effects of DOR ligands involve monoamines. Thus, SNC80-induced hypothermia in rats was abolished by pre-treatment with naltrindole, attenuated by pre-treatment with the selective 5-HT_{1A} receptor antagonist WAY100635 and enhanced by pre-treatment with the selective 5-HT reuptake inhibitor (SSRI) fluoxetine (Rawls and Cowan 2006). Spina and colleagues (Spina et al. 1998) reported that SNC80 and another non-peptide DOR agonist BW373U86, induced locomotion, rearing, stereotyped sniffing, licking and gnawing in rats. These behavioural effects were abolished by pre-treatment with naltrindole and reduced by pre-administration of dopamine D_2/D_3 receptor antagonist raclopride.

Thirdly, the modulatory effect of the DORs on brain monoamine concentrations was observed. In olfactory bulbectomized rats, an animal model of depression, SNC80 increased extracellular 5-HT concentrations in the prefrontal cortex, hippocampus, and amygdala, to the levels observed in control animals (Saitoh et al. 2008). Bosse and co-authors reported that SNC80 enhances amphetamine-mediated efflux of dopamine from rat striatum (Bosse et al. 2008).

Bases on the abovementioned lines of evidence, we hypothesize that SNC80 and naltrindole alter the excitability of hippocampal neurons in *in vivo* conditions. The effects of these DOR ligands on *in vivo* excitability of hippocampal neurons might be direct, as well as mediated *via* monoaminergic circuits. Our present study aims to test this hypothesis.

Materials and Methods

Animals

Adult male Wistar rats (250–300 g) were ordered from the Animal Breeding facility of the Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences (Dobra Voda, Slovakia). Animals were housed under standard laboratory conditions (temperature: 22 ± 2°C, humidity: 55 ± 10%) with a 12 h light/12 h dark cycle (lights on at 7 a.m.). Pelleted food and tap water were available *ad libitum*. All experimental procedures were approved by the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic (Permit number Ro 3592/15-221) and conformed to the Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes. In this type of investigation, the use of animals could not be avoided (Homberg et al. 2021).

Chemicals

SNC80 and naltrindole were ordered from Bio-Techne Ltd., Abingdon, UK. Other chemicals were purchased from Merck Life Science s.r.o, Bratislava, Slovakia. Chloral hydrate, urethane, and naltrindole were dissolved in saline (0.9% sodium chloride: NaCl in water). SNC80 was dissolved in 1 M hydrochloric acid (HCl). The SNC80 solution in HCl was subsequently diluted with saline (1:100) and titrated with sodium hydroxide (NaOH) to pH ≈ 7.

In vivo electrophysiological experiments

In vivo electrophysiological experiments were performed as previously described (Csatlosova et al. 2021; Dremencov et al. 2022; Paliokha et al. 2022). Animals were anesthetized by urethane (1.25 g/kg, intraperitoneally (i.p.), for the assessment of excitability of hippocampal glutamate neurons) or chloral hydrate (0.4 g/kg, i.p., for the assessment of excitability of brainstem monoamine neurons) and mounted in the stereotaxic frame (David Kopf Instruments, Tujunga, CA). Body temperature was maintained between 36 and 37°C with a heating pad (Gaymor Instruments, Orchard Park, NY, USA).

The scalp was opened, and a 3 mm hole was drilled in the skull for insertion of electrodes. Glass-pipettes were pulled with a DMZ-Universal Puller (Zeitz-Instruments GmbH, Martinsried, Germany) to a fine tip approximately 1 μm in diameter and filled with 2 M NaCl solution. Electrode impedance ranged from 4 to 6 MΩ. The pipettes were inserted into the cornu ammonis 1/3 (CA1/3) area of the hippocampus (3.9–4.2 mm posterior to bregma, 2.2–2.8 mm lateral to the midline, and 1.9–3.5 mm ventral to brain surface), DRN (7.8–8.3 mm posterior to bregma and 4.5–7.0 mm ventral to brain surface), LC (8.0–8.3 mm posterior to bregma, 1.2–1.4 mm lateral to the midline, and 5.5–7.5 mm ventral to the brain surface), or VTA (4.5–5.5 mm posterior to bregma, 0.6–0.8 mm lateral to the midline, and 7.0–8.5 mm ventral to the brain surface) (Paxinos and Watson 2014) by hydraulic micro-positioner (David Kopf Instruments, Tujunga, CA).

The action potentials generated by monoamine-secreting neurons were recorded using the AD Instruments Extracellular Recording System (Dunedin, New Zealand). Pyramidal CA1/3 neurons were identified based on the following criteria: large amplitude (0.5–1.2 mV), long-duration (0.8–1.2 ms) simple action potentials alternating with complex spike discharges (Fig. 1A) (Kandel and Spencer 1961; El Mansari et al. 2020). The 5-HT DRN neurons were identified by bi- or tri-phasic action potentials with a rising phase of long duration (0.8–1.2 ms) and regular firing rate of 0.5–5.0 Hz (Fig. 1B). Noradrenergic LC neurons were recognized by action potentials with a long-duration rising phase (0.8–1.2 ms), regular firing rate of 0.5–5.0 Hz, and a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw (Fig. 1C) (Vandermaelen and Aghajanian 1983). Dopaminergic VTA neurons were recognized by tri-phasic action potentials lasting between 3 and 5 ms with a rising phase lasting over 1.1 ms, inflection or "notch" during the rising phase, marked negative deflection, irregular firing-rate of 0.5–10 Hz, mixed single-spike and burst firing with characteristic decrease of the action potentials amplitude within the bursts (Fig. 1D) (Grace and Bunney 1983).

SNC80 and naltrindole administration

SNC80 and naltrindole were administered, *via* a catheter placed in a femoral vein, after a CA1/3 glutamate ($n = 15$ neurons from 15 rats), DRN 5-HT ($n = 13$ neurons from 13 rats), LC noradrenaline ($n = 10$ neurons from 10 rats), or VTA dopamine ($n = 12$ neurons from 12 rats) neuron was identified and its basal firing activity was recorded for 2 min, at cumulative doses of 1–3 mg/kg each. The number of animals was limited to the needed to obtain statistically meaningful data. Different number of animals was required to obtain statistically

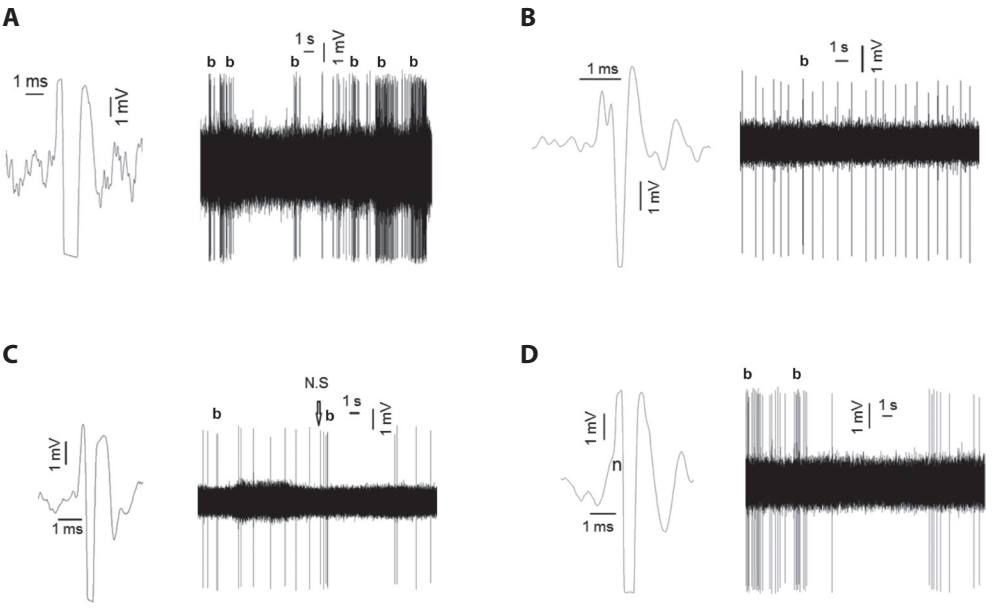

Figure 1. Identification of glutamate neurons of the *cornu ammonis* 1/3 (CA1/3) of hippocampus (**A**), serotonin (5-HT) neurons of the dorsal raphe nucleus (DRN; **B**), norepinephrine neurons of the locus coeruleus (LC; **C**), and dopamine neurons of the ventral tegmental area (VTA; **D**). NS, contralateral nociceptive stimuli; n, notch; b, burst.

meaningful data from glutamate, 5-HT, noradrenaline, and dopamine neurons, because of the different variability in the excitability of these neurons. The doses were chosen according to the previous behavioral studies (Jutkiewicz et al. 2003, 2005a; Saitoh et al. 2004). In the first series of experiments, SNC80 was administered first (three consecutive injections of 1 mg/kg each), and naltrindole was injected (three times, 1 mg/kg each) afterward. In the second series of experiments, naltrindole was administered first (three consecutive injections of 1 mg/kg each), and SNC80 was injected subsequently (three times, 1 mg/kg each). The minimal interval of 2 min was preserved between two different injections.

Statistical analysis

The effects of acute SNC80 and naltrindole on the excitability of hippocampal glutamate and brainstem monoamine neurons, the spikes frequency after the administration of each dose of SNC80 or naltrindole was expressed as percentage of the basal firing activity of the same neurons. The effects of SNC80 or naltrindole were examined using the analysis of variance for repeated measures (RM ANOVA), followed by Bonferroni *post-hoc* test.

Result

Acute SNC80 administration inhibited hippocampal CA1/3 glutamate neurons; naltrindole prevented SNC80-induced inhibition of hippocampal CA1/3 glutamate neuronal activity

Acute SNC80, administered first, significantly (comparing to the baseline) and dose-dependently inhibited CA1/3 glutamate neurons, from 80% of baseline after the administration of 1 mg/kg of SNC80 to the maximum of 50% of baseline after the administration of 3 mg/kg of SNC80; the doses chosen according to the previous behavioral studies (Jutkiewicz et al. 2003, 2005a; Saitoh et al. 2004); subsequent injection of naltrindole did not have any additional effect ($F_{6,41} = 2.77$, $p < 0.05$, RM ANOVA, data from 6 neurons from 6 animals; Fig. 2A). Acute naltrindole did not alter the firing rate of CA1/3 glutamate neurons; neither did SNC80 which was injected consecutively (data from 9 neurons from 9, animals; Fig. 2B).

Acute SNC80 administration inhibited, and acute naltrindole administration stimulated 5-HT neurons of the DRN; naltrindole-induced stimulation of 5-HT neurons was reversed by SNC80

Acute SNC80, administered first, significantly (comparing to the baseline) and dose-dependently inhibited DRN 5-HT neurons, from 80% of baseline after the administration of 1 mg/kg of SNC80 to the maximum of 30% of baseline after the administration of 3 mg/kg of SNC80 ($F_{6,34} = 3.63$, $p < 0.05$, RM ANOVA, data from 5 neurons from 5 animals; Fig. 3A). Subsequent injection of naltrindole did not have any additional effect on DRN 5-HT neuronal firing activity. When it was injected first, acute naltrindole significantly (comparing to the baseline) and dose-dependently stimulated DRN 5-HT neurons, from 125% of baseline after the administration of 1 mg/kg of naltrindole to the maximum of 250% of baseline after the administration of 3 mg/kg of naltrindole; subsequent SNC80 reversed naltrindole-induced stimulation in a dose-dependent manner, to 150% of baseline after the administration of 1 mg/kg of SNC80 and to the minimum of 30% of baseline after the administration of 3 mg/kg of SNC80 ($F_{6,55} = 2.53$, $p < 0.05$, RM ANOVA, data from 8 neurons from 8 animals; Fig. 3B).

Co-administration of SNC80 and naltrindole, but neither of them alone, attenuated the firing activity of noradrenaline neurons of the LC

Acute SNC80, administered first, did not alter the firing activity of LC noradrenaline neurons in a statistically significant way. The subsequent administration of 3 mg/kg of naltrindole inhibited the firing of norepinephrine neurons to the 5% of baseline ($F_{1,9} = 707.56$, $p < 0.001$, RM ANOVA, data from 5 neurons from 5 animals, comparison between 3 mg/kg SNC80 + 3 mg naltrindole and baseline only; Fig. 4A). Naltrindole, administered first, tended to stimulate LC noradrenaline neurons, and subsequent administration of SNC80 tended to reverse this stimulation, but these effects were not statistically significant (comparing to the baseline; data from 5 neurons from 5 animals; Fig. 4B).

Effect of acute SNC80 and naltrindole administration on the excitability of VTA dopamine neurons

Acute SNC80, administered first, significantly (comparing to the baseline) and dose-dependently inhibited VTA dopamine neurons, from 50% of baseline after the administration of 1 mg/kg of SNC80 to the maximum of 30% of baseline after the administration of 3 mg/kg of SNC80; subsequent injection of naltrindole did not have any additional effect ($F_{6,48} = 5.13$, $p < 0.001$, data from 7 neurons from 7 animals; Fig. 5A). Acute naltrindole tended to stimulate VTA dopamine neurons, and subsequent administration of SNC80 tended to reverse this stimulation, but these effects were not statistically significant (data from 5 neurons from 5 animals; Fig. 5B).

Discussion

In the present study we tested, for the first time, the effect of the selective DOR agonist SNC80 and selective DOR antagonist

naltrindole on the excitability of the individual hippocampal glutamate and brainstem monoamine neurons in *in vivo* conditions. The results of the present study suggest that acute administration of SNC80 had an inhibitory effect on the excitability on hippocampal glutamate and brainstem 5-HT and dopamine neurons. The acute inhibitory effects of SNC80 on the hippocampal CA1/3 glutamate and brainstem DRN 5-HT and VTA dopamine neurons were prevented by pre-treatment with naltrindole, suggesting that these effects are DOR-mediated.

The inhibitory effect of SNC80 on the CA1/3 glutamate, DRN 5-HT, and VTA dopamine neurons was dose-dependent. Partial inhibition of the neuronal firing activity (80% of baseline in glutamate and 5-HT and 50% of baseline in dopamine neurons) was observed after the administration of 1 mg/kg of SNC80, and the maximal inhibition (30% of baseline) was recorded after the administration of 3 mg/kg of SNC80. Maximal neurophysiological effect, observed after 3 mg/kg of SNC80, is consistent with the maximal neurotheological effects observed after the administration of the same DOR ligand at the same dose (Jutkiewicz et al. 2003, 2005a; Saitoh et al. 2004). It is thus possible that the maximal occupation of the DOR receptors by SNC80 in the rat brain is achieved at this dosage.

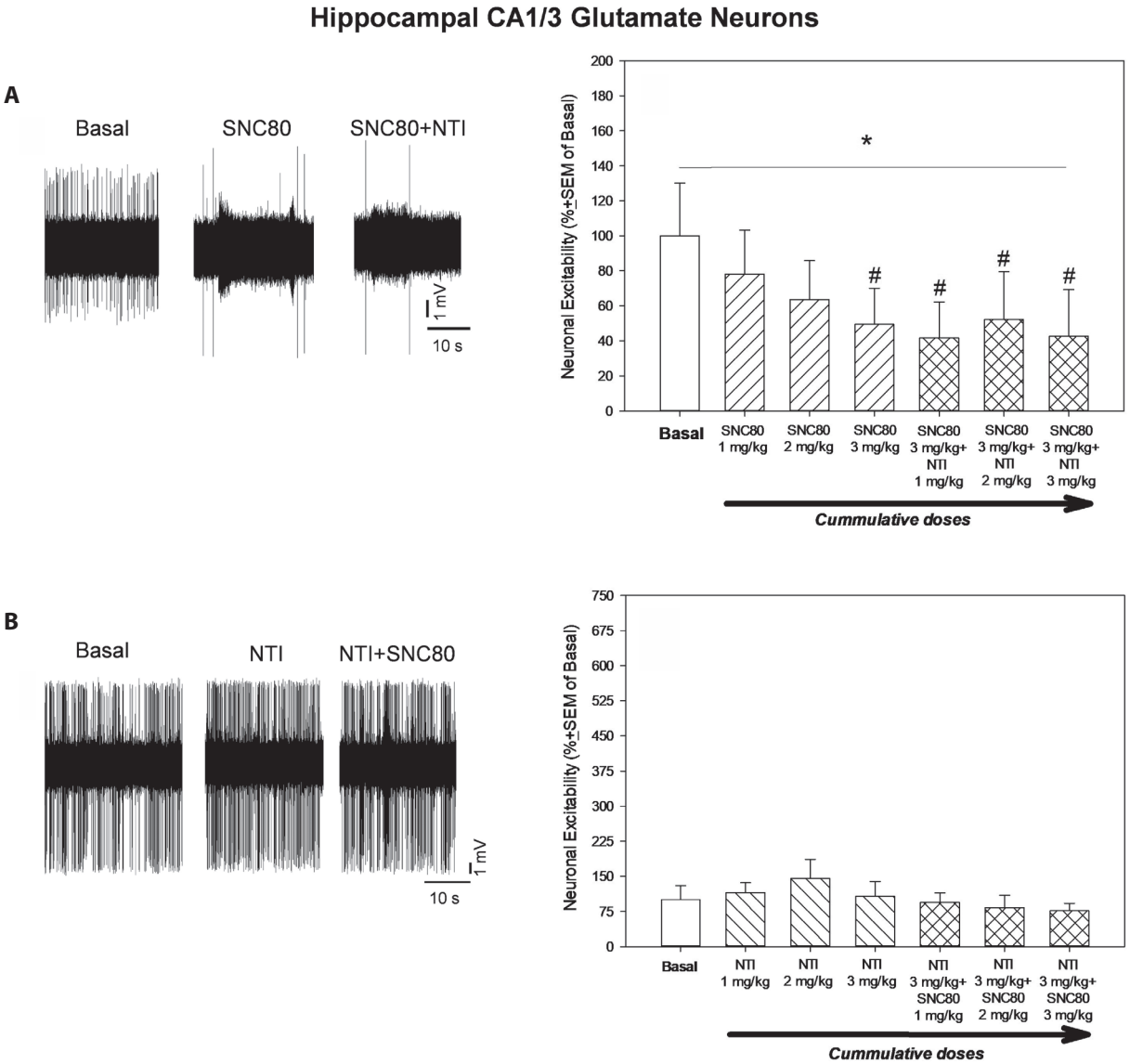

Figure 2. Effect of acute SNC80 (**A**) and naltrindole (NTI; **B**) administration on the excitability of hippocampal cornu ammonis 1/3 (CA1/3) glutamate neurons. SNC80 administration was followed by NTI administration (A). NTI administration was followed by SNC80 administration (B). Left: representative recordings from the individual CA1/3 glutamate neurons. Right: summary effect from 6 neurons of 6 animals (A) and 9 neurons from 9 animals (B). * $p < 0.05$, RM ANOVA; # $p < 0.05$ in comparison with baseline, Bonferroni *post-hoc* test. Data are mean ± SEM.

Since SNC80 and naltrindole were administered systemically, their effects on the firing activity of hippocampal glutamate and midbrain 5-HT, noradrenaline, and dopamine neurons do not necessary result from the activation of the DORs in the CA1/3, DRN, LC, and VTA, respectively. DORs expressed in other brain areas (e.g., prefrontal cortex : PFC) projecting to the hippocampus and/or monoaminergic nuclei (Hajós et al. 1999) can be involved as well. Although SNC80 and naltrindole have relatively high selectivity to the DORs, the ability of these ligands to target other molecules, such as voltage-dependent sodium channels (VDCCs) (Remy et al. 2004), can contribute their effect on hippocampal glutamate and midbrain neurons as well. Finally, heterodimerization between DORs and μ-ORs (MORs) and the ability of SNC80 to activate MOR-DOT heteromeric receptors can be involved as well (Metcalf et al. 2012).

We found that acute SNC80 inhibited hippocampal glutamate neurons in *in vivo* conditions. Interestingly, in the isolated hippocampal neurons in primary cultures SNC80 had an opposite, stimulatory effect (Moravcikova et al. 2021). Our finding also allegedly contradicts to the opioid-induced, γ-aminobutyric (GABA)-interneuron-mediated stimulation of hippocampal pyramidal cells, suggested by Zieglgänsberger and colleagues (Zieglgänsberger et al. 1979). It is thus possible

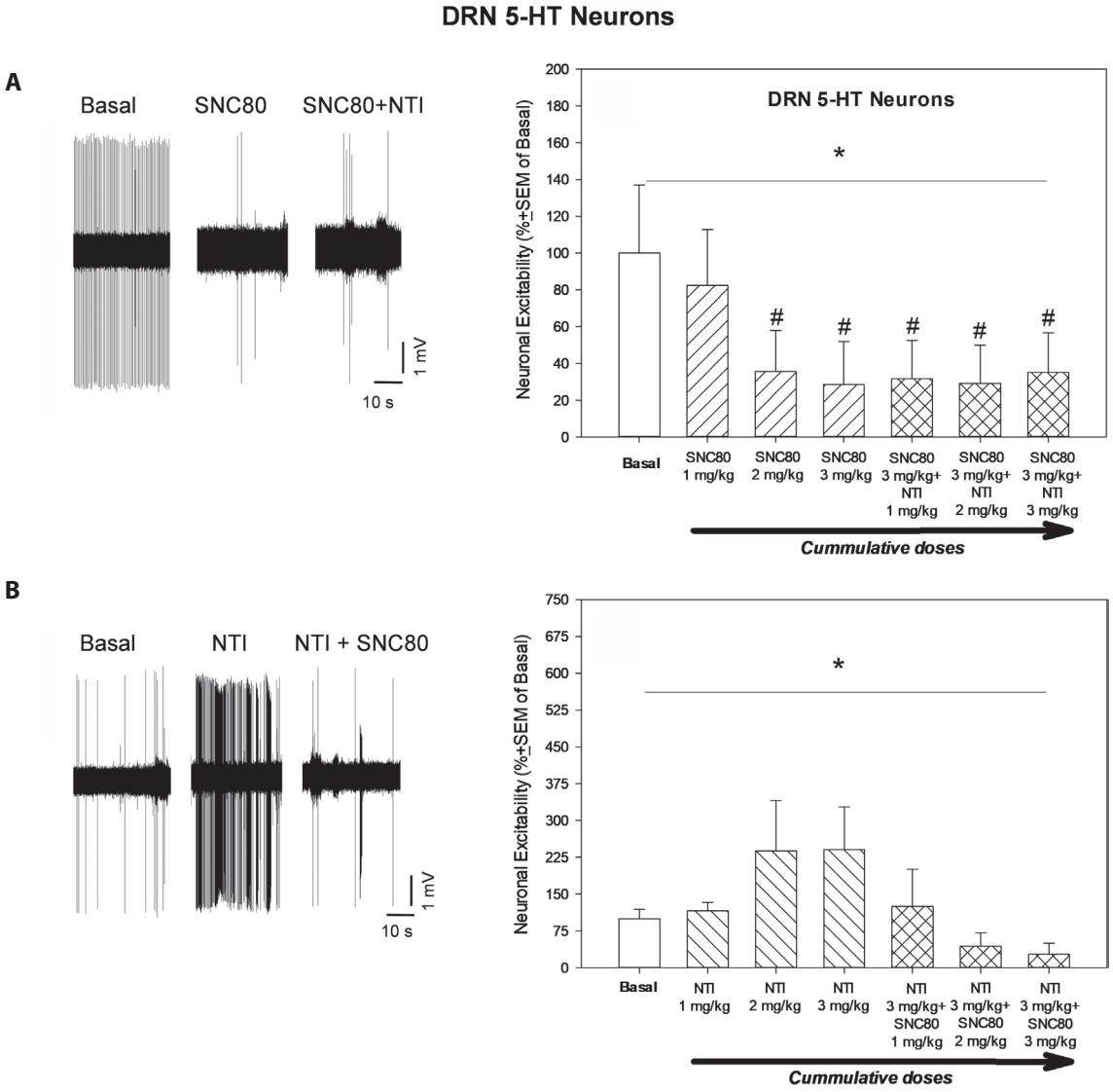

Figure 3. Effect of acute SNC80 (**A**) and naltrindole (NTI; **B**) administration on the excitability of dorsal raphe nucleus (DRN) serotonin (5-HT) neurons. SNC80 administration was followed by NTI administration (A). NTI administration was followed by SNC80 administration (B). Left: representative recordings from the individual DRN 5-HT neurons. Right: summary effect from 5 neurons from 5 animals (A) and 8 neurons from 8 animals (B). * $p < 0.05$, RM ANOVA; # $p < 0.05$ in comparison with baseline, Bonferroni *post-hoc* test.

that another neurotransmitter, such as 5-HT, is involved in SNC80-inuced inhibition of hippocampal glutamate neurons in *in vivo* conditions. It was indeed found that SNC80 raised extracellular 5-HT levels in the hippocampus (Saitoh et al. 2008). Hippocampal 5-HT is known to inhibit glutamate neurons, *via* a mechanism involving 5-HT_{1A} receptors (Dong et al. 1997; Herman et al. 2018). It is likely that this 5-HT_{1A}-mediated inhibitory effect of acute SNC80 on hippocampal CA1/3 glutamate neurons overpowers the direct DOR-mediated excitatory effect of this ligand, which was observed *in vitro*.

We found that acute naltrindole dose-dependently stimulated 5-HT neurons of the DRN. This naltrindole-induced stimulation of DRN 5-HT neurons was completely reversed by SNC80, in a dose-dependent manner. It is thus possible that DORs expressed in the DRN are tonically activated by endogenous opioids, such as β-endorphin. Expression of postsynaptic DORs in the DRN (Wang et al. 1997) and innervation of the DRN by β-endorphin secreting neurons of the arcuate nucleus (Veening et al. 2012) partially support this hypothesis.

When acute naltrindole was administered after acute SNC80, it failed to reverse SNC80-induced inhibition of hippocampal glutamate and brainstem 5-HT and dopamine neurons. Acute SNC80 administered after naltrindole,

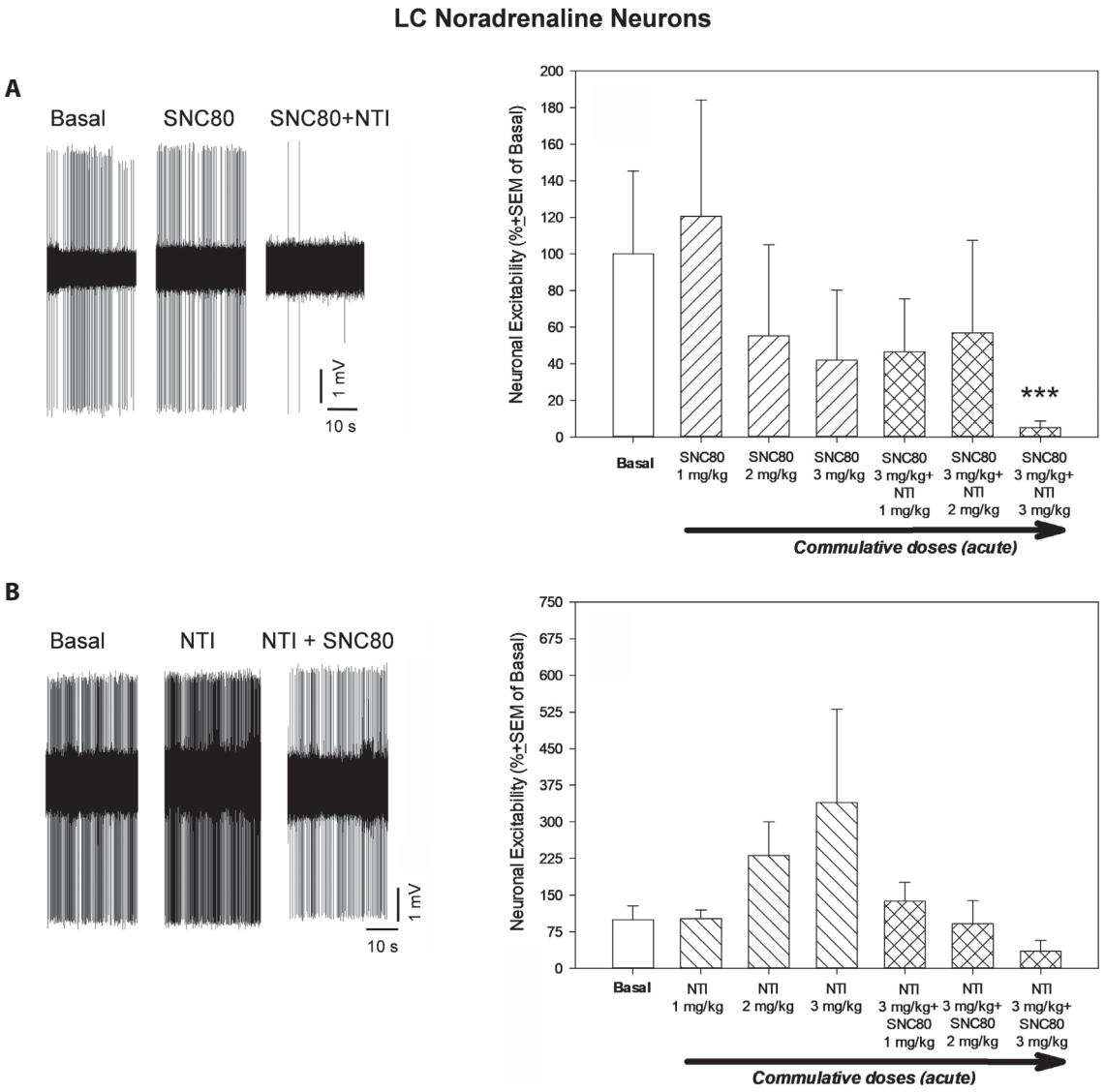

Figure 4. Effect of acute SNC80 (**A**) and naltrindole (NTI; **B**) administration on the excitability of locus coeruleus (LC) noradrenaline neurons. SNC80 administration was followed by NTI administration (A). NTI administration was followed by SNC80 administration (B). Left: representative recordings from the individual LC noradrenaline neurons. Right: summary effect from 5 neurons from 5 animals (A) and 5 neurons from 5 animals (B). *** $p < 0.001$, RM ANOVA.

however, reversed naltrindole-induced inhibition of DRN 5-HT neurons. It is this likely that the functional affinity of SNC80 to the DORs expressed on the hippocampal glutamate and brainstem 5-HT and dopamine neurons is higher than this of naltrindole. Remarkably, the binding affinity of naltrindole to the recombinant rat DORs expressed on isolated membranes is higher than this of SNC80 (Maguire and Loew 1996). The functional efficacies of SNC80 and naltrindole to the DORs, evaluated as the ability of these compounds to alter cAMP synthesis in brain slices, are however similar (Jutkiewicz et al. 2005b; Tanguturi et al. 2021). It is possible that in *in vivo* conditions, and/or on in sub-population of the DORs expressed in the DRN, SNC80 has higher functional efficacy than naltrindole. Alternatively, the washout time of SNC80 in *in vivo* conditions is higher than that of naltrindole.

Interestingly, statistically significant inhibitory effect of SNC80 and naltrindole on the excitability of LC noradrenaline neurons was detected only when both ligands were co-administered, and only when SNC80 was administered prior to naltrindole. This result is in some way consistent with the outputs of our previous study (Moravcikova et al. 2021), which showed that the effects of SNC80 and naltrindole on neuronal excitability might not be always opposite.

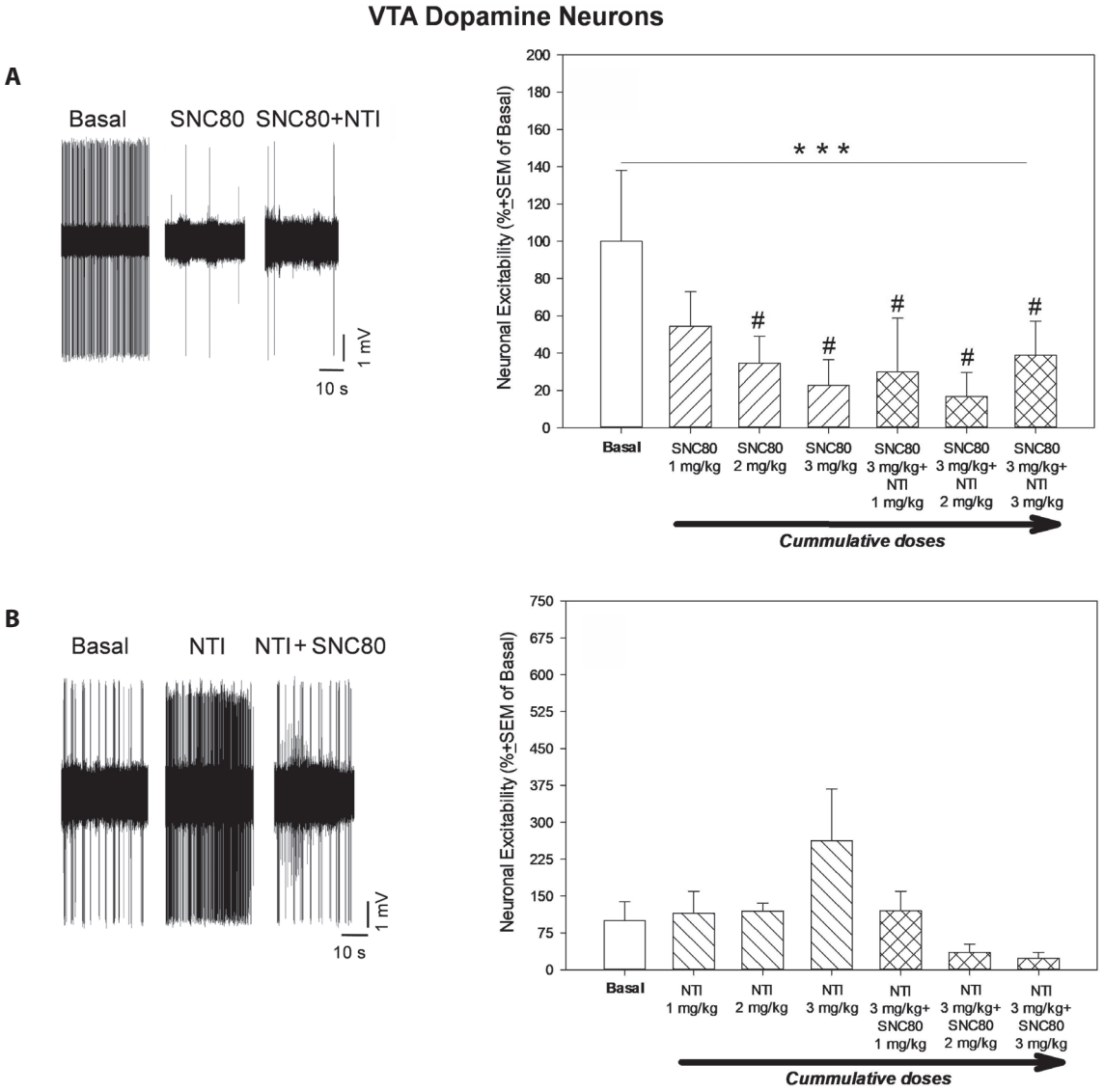

Figure 5. Effect of acute SNC80 (**A**) and naltrindole (NTI; **B**) administration on the excitability of ventral tegmental area (VTA) dopamine neurons. SNC80 administration was followed by NTI administration (A). NTI administration was followed by SNC80 administration (B). Left: representative recordings from individual VTA dopamine neurons. Right: summary effect from 7 neurons from 7 animals (A) and 5 neurons from 5 animals (B). * $p < 0.05$, RM ANOVA; # $p < 0.05$ in comparison with baseline, Bonferroni *post-hoc* test.

Thus, SNC80 and naltrindole exert opposite effects on the DOR-$G_{I/O}$ protein-adenylyl cyclase (AC)-cyclic AMP (cAMP)-voltage-dependent sodium channels (VDSCs) pathway, resulting in the reverse (stimulatory and inhibitory, respectively) effects on the spontaneous activity of cultivated neurons. However, SNC80 also exerts a DOR-independent inhibitory effect on the VDSCs (Remy et al. 2004), and naltrindole-on the voltage-dependent calcium channels (VDCCs), resulting in a similar excitatory effects of these ligands on the depolarization pulse-triggered activity of cultivated neurons (Moravcikova et al. 2021). Since VDSCs and VDCCs both contribute to the generation of the action potentials in noradrenaline neurons (von Kügelgen et al. 1999), it is thus possible that the inhibition of noradrenaline neurons of the LC can be achieved after the simultaneous inhibition of VDSCs and VDCCs by SNC80 and naltrindole, respectively. The fact that DORs are poorly expressed in the LC, comparing to the solid expression of these ORs in the DRN and VTA (Erbs et al. 2015); partially support our suggestion that SNC80 and naltrindole effects on noradrenaline neurons of the LC are not DOR-mediated. Further studies are needed to examine this hypothesis.

In conclusion, the results of the present study suggest that acute administration of DOR agonist leads to the inhibition of hippocampal glutamate and brainstem 5-HT and dopamine neurons. The inhibitory effect of DOR agonists on hippocampal glutamate neurons putatively involves increase in brain 5-HT concentrations and activation of 5-HT_{1A} receptors. The ability to modulate the excitability of hippocampal glutamate and brainstem 5-HT and dopamine neurons may be involved in anxiolytic and antidepressant-like effects of the DOR agonists. Further studies, e.g., testing of the effect of chronic treatment with DOR ligands on the hippocampal glutamate and brainstem 5-HT and dopamine neurons, are required to test this hypothesis.

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Conflicts of interest. The authors declare no conflict of interest.

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