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Transforming growth factor beta 1 (TGF-β1) modulates central noradrenergic neurotransmission via a TGF-β receptor 1 (TGF-βR1)-mediated mechanism

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Research Article

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Abstract

Background

Transforming growth factor-β1 (TGF-β1) signaling has been implicated in neuropsychiatric disorders, but its direct electrophysiological effects on central monoaminergic neurons remain unclear. This study examined whether TGF-β1 modulates serotonergic, dopaminergic, and noradrenergic neuronal activity in vivo.

Methods

Adult rats received acute TGF-β1 administration, and extracellular single-unit recordings were performed from serotonergic neurons in the dorsal raphe nucleus (DRN), dopaminergic neurons in the ventral tegmental area (VTA), and noradrenergic neurons in the locus coeruleus (LC). Additional experiments assessed the involvement of noradrenergic neurotransmission using fusaric acid-induced noradrenaline depletion and the role of TGF-β receptor type I (TGF-βR1) using the antagonist LY2109761.

Results

TGF-β1 did not significantly alter the firing activity of DRN serotonergic neurons or VTA dopaminergic neurons. In contrast, TGF-β1 significantly reduced the spontaneous firing activity and burst parameters of LC noradrenergic neurons. Noradrenaline depletion with fusaric acid abolished the inhibitory effect of TGF-β1 on LC neuronal activity. Conversely, pharmacological blockade of TGF-βR1 with LY2109761 increased LC neuronal firing, suggesting that endogenous TGF-β1 signaling exerts a tonic inhibitory influence on the noradrenergic system.

Conclusion

These findings identify TGF-β1/TGF-βR1 signaling as a selective modulator of central noradrenergic neuronal activity. The noradrenaline-dependent suppression of LC firing may represent a mechanism linking TGF-β1 signaling with monoaminergic circuits involved in stress regulation and affective disorders.

Introduction

Transforming growth factor beta 1 (TGF-β1), a member of the cytokine superfamily of transforming growth factors, plays a crucial role in various biological processes, including immune response [1], cell proliferation (Massagué [2], and differentiation [3]. Beyond its primary functions within the immune systems, TGF-β1 plays an important role in the central nervous system (CNS). Modulating the neuroinflammatory responses through this cytokine is crucial, as evidenced by altered levels of TGF-β1

in patients with depression [4, 5]. This alteration suggests a potential link between dysregulation of TGF-β1 and the manifestation of depressive symptoms [6]. TGF-β1 has the capacity to exert both activating and repressive effects on cell proliferation, depending on the specific cell type and environment in which it operates. TGF-β1 signaling occurs through a complex series of intracellular pathways that can be broadly categorized into canonical (Smad-dependent) and non-canonical (Smad-independent) pathways [7].

Several lines of evidence suggest a potential link between impaired TGF-β1 signaling and cognitive deficits associated with depression. In animal models of depression, which also exhibit cognitive impairments, disruptions in TGF-β1 signaling have been identified as a contributing factor to these deficits [8]. Long-term administration of fluoxetine or the novel multimodal antidepressant vortioxetine completely alleviated depression-related characteristics and memory impairments in mice injected with amyloid beta, the main component of amyloid plaques, extracellular deposits found in the brains of people with Alzheimer's disease. This phenomenon was accompanied by the restoration of TGF-β1 levels in the hippocampus [9]. Dobolyi et al. demonstrated that TGF-β1 is dynamically expressed in the adult brain and is markedly upregulated in response to injury and inflammatory stimuli. The study showed that TGF-β1 is produced by both neurons and glial cells and plays a neuroprotective role by modulating inflammation, promoting cell survival, and contributing to tissue repair processes [10]. Sometani and colleagues reported that TGF-β1 enhanced BDNF expression in cultured cortical neurons, partly through TGF-β1’s neurotrophic activity [11].

Using RNA sequencing in the prefrontal cortex (PFC) of mice exposed to chronic social defeat stress (CSDS), Zhang and colleagues found that TGF-β1 plays a role in the antidepressant-like effects of arketamine [12]. Intracerebroventricular injections of recombinant TGF-β1 conducted by the same team also induced rapid and long-lasting antidepressant-like effects in CSDS models [12]. The most intriguing finding, however, may be that even intranasal administration of TGF-β1 triggered rapid and sustained antidepressant-like effects in the CSDS model [13].

Intranasal administration represents a promising strategy for delivering therapeutics to the CNS, particularly for biologically unstable molecules whose systemic delivery is limited by the blood–brain barrier (BBB). By enabling direct transport along olfactory and trigeminal pathways, this route bypasses the BBB and enhances brain bioavailability [14]. In rodents, intranasally administered TGF-β1 reached multiple brain regions, with the highest levels detected in the olfactory bulb, and modulated the in vivo expression of its type 1 (TGF-β1R1) and 2 (TGF-β1R2) receptors, confirming functional activity within the CNS [15, 16].

It is well established that dopaminergic, noradrenergic and serotonergic (5-HT) systems of the brain are fundamental in pathophysiology and treatment of depression. Most of the known antidepressant drugs act on monoaminergic systems of the brain [17]. Our study aimed to test the hypothesis that the antidepressant-like effect of TGF-β1 is mediated, at least in part, *via* the modulation of the excitability of

the central dopaminergic, noradrenergic and 5-HT neurons. Another goal of the study was to identify specific receptor(s) involved in the interactions between TGF-β and central monoaminergic systems.

Material and Methods

Animals

Adult male Wistar rats (body weight: 250–350 g) were obtained from the Institute of Toxicology and Breeding of Laboratory Animals, Centre of Experimental Medicine, Slovak Academy of Sciences (Dobrá Voda, Slovakia). The animals were housed in the central animal facility of the Slovak Academy of Sciences, Medical Sciences Pavilion. Rats were kept in plastic cages (50 × 40 × 20 cm; maximum of 5 rats per cage) under standard laboratory conditions (temperature: 22 ± 2°C, humidity: 55 ± 10%), with a 12 h light/12 h dark cycle (lights on at 7:00 a.m.), and had *ad libitum* access to food and water. Animals were allowed to acclimatize for at least one week prior to the start of the experiments. All experimental procedures were approved by the State Veterinary and Food Administration of the Slovak Republic (Permit No 732/2022 from January 25, 2022) and were conducted in accordance with Directive 2012/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

Chemicals

TGF-β1 protein was purchased from Molchem s.r.o. (Pri Sypke 12, Michalovce, Slovakia). For stock preparation, 10 µL of TWEEN-80 was added to 100 mL distilled water, and the solution was stored at 4°C. Subsequently, 1 mL of TWEEN-80/DW was added to the vial containing 1 mg TGF-β1, resulting in a stock solution of 1 mg/mL. The stock was aliquoted into four 250 µL portions and stored at − 20°C until use. For intranasal administration, 10 µL of the TGF-β1 stock solution was diluted in 1000 µL physiological saline (0.9% NaCl) to obtain the working solution, which was prepared fresh prior to use. Fusaric acid (≥ 99%; Carl Roth, Germany) was prepared for intraperitoneal administration as a 50 mg/mL stock solution in 0.9% saline by dissolving 0.5 g in 10 mL. The selective antagonist of TGF-β1R1, LY2109761, (Lambda Life a.s., Bratislava, Slovakia) was prepared as a 5 mM solution and administered at a dose of 25 nmol in 5 µL. The compound vehicle consisted of 10% DMSO, 40% PEG-300, 5% Tween-80, and 45% water.

Intranasal administration of TGF-β1

At the beginning of the experiment, adult rats were weighed. They were then placed into a chamber connected to an anesthesia apparatus (Parkland Scientific, Coral Springs, FL, USA) and anesthetized using isoflurane (Isoflurin®, Vetpharma, Barcelona, Spain). Immobilized rats were administered physiological saline (0.9% NaCl) or TGF-β1 (0.01 mg/kg) into each nostril using a micropipette, with the animals held in an almost horizontal position, lying on their backs, while the solutions were dripped directly into the nasal cavity. A 5-minute interval was maintained between administration into the first

and second nostril. After completion of this part of the experiment, the rats were returned to their cages for a period of 60 minutes.

Noradrenaline depletion by fusaric acid

Noradrenaline synthesis inhibition by fusaric acid was used to evaluate the role of extracellular noradrenaline in the TGF-β1-mediated modulation of the firing activity of noradrenergic neurons. The acid was administered by two intraperitoneal (i.p.) injections 6 and 4 hours before the electrophysiological assessments, as previously described [18].

TGF-βR1 blockade by of LY2109761

Animals were anesthetized with chloral hydrate (0.4 g/kg, i.p.) and placed within the stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Body temperature was maintained at 37°C using a heating pad (Gaymor Instruments, Orchard Park, NY, USA). Additional doses of chloral hydrate were administered when necessary. The skull was opened and a 2-mm hole was drilled in the scull above the left lateral cerebral ventricle (− 0.8 to − 1.0 mm posterior to bregma, ± 1.4 to ± 1.6 mm lateral to the midline,[19], using the David Kopf NH-180 high-speed stereotaxic drill (38,000 RPM). The needle of a Hamilton micro-syringe (Hamilton Company, Reno, NV, USA) was inserted into the ventricle (3.5 ventral to the brain surface). LY2109761 (25 nmol in 5 µL) was administered via intracerebroventricular injection. The animals remained anesthetized and secured in the stereotaxic frame, and electrophysiological recordings were performed 1 hour after administration.

In vivo electrophysiology

In the rats pre-treated with LY2109761, an additional hole was drilled in the scull above the dorsal raphe nucleus (DRN; 8 mm posterior to the bregma), ventral tegmental area (VTA; 5 mm posterior to bregma and 0.6 mm lateral to midline), or locus coeruleus (LC; 8.1 mm posterior to bregma and 1.1 mm lateral to midline [19]. Other rats were anesthetized with chloral hydrate and mounted in the stereotaxic frame, as previously explained; the hole above the DRN, VTA, or LC was drilled thereafter. Glass borosilicate electrodes were pulled using a DMZ Universal Puller (Zeitz-Instruments GmbH, Martinsried, Germany) and filled with 2 M NaCl solution. The recorded signal was amplified using a DP-311 differential amplifier (AD Instruments, Dunedin, New Zealand), filtered from electrical noise using a HumBug noise eliminator (Carlsborg, WA, USA), and digitized via a 4/35 PowerLab data acquisition system connected to a personal computer (Lenovo 1010, SAR Hong Kong, China) using LabChart 7.5 software (AD Instruments, Dunedin, New Zealand). The sampling rate was set to be 100 kHz.

The 5-HT neurons were recorded from the DRN at coordinates 7.8–8.3 mm posterior to bregma and 4.5–7.0 mm ventral to the brain surface [19]. These neurons were identified based on a regular firing rate of 0.5–5.0 Hz and long-duration positive action potential (0.8–1.2 ms) [20, 21].

Dopaminergic neurons were recorded from the VTA at coordinates 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 [19]. Dopamine neurons were identified by their slow, irregular firing rate (0.5–10.0 Hz), mixed single-spike and burst firing pattern, tri-phasic action potentials with a dominant positive component exceeding 2.5 ms, and the presence of a characteristic “notch” at the beginning of the rising phase [20 1983].

Noradrenergic neurons were recorded from the LC at coordinates 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 [19]. These neurons were identified by their regular firing rate (0.5–10.0 Hz), long-duration positive action potentials (0.8–1.2 ms), and a characteristic burst response to nociceptive pinch of the contralateral hind paw [22].

Statistical analysis

Action potentials or “spikes” of 5-HT, dopaminergic, and noradrenergic neurons were detected using the spike-sorting algorithm Spike2 (Cambridge Electronic Design, Cambridge, UK). Neuronal firing rate and burst activity were calculated using the burstiDator software (www.github.com/nno/burstidator). For analysis of data from experiments involving no more than two groups of subjects Student's t-test was used. The probability value of $p \leq 0.05$ was considered statistically significant. Statistical analyses were performed using SigmaPlot 12.5 (Systat Software Inc., Chicago, IL, USA).

Results

TGF-β1 did not alter the excitability of dopaminergic of the VTA or serotonergic neurons of the DRN

Figure 1 illustrates the effect of intranasal TGF-β1 administration on the excitability of serotonergic neurons of the DRN. Representative extracellular single-unit recordings are shown in panels A (vehicle) and B (TGF-β1). Regarding the mean neuronal firing rate (C), no statistically significant difference was observed between vehicle- and TGF-β1-treated rats. Similarly, analysis of burst frequency (D) revealed no significant effect of TGF-β1 treatment. With respect to the percentage of spikes occurring within bursts (E), no statistically significant difference was detected between groups. The mean number of spikes per burst (F) also did not differ between vehicle- and TGF-β1-treated animals. No effect on the mean number of the spontaneously active neurons per electrode track was observed (not shown).

Figure 2 illustrates the effect of intranasal TGF-β1 administration on the excitability of dopaminergic neurons of the VTA. Representative extracellular single-unit recordings are shown in panels A (vehicle) and B (TGF-β1). With respect to the mean neuronal firing rate (C), no statistically significant difference was observed between vehicle- and TGF-β1-treated animals. Similarly, burst frequency (D) did not differ between groups. Analysis of the percentage of spikes occurring within bursts (E) also revealed no significant difference between vehicle- and TGF-β1-treated rats. The mean number of spikes per burst (F) remained unchanged following TGF-β1 administration. No effect on the mean number of the spontaneously active neurons per electrode track was observed (not shown).

TGF-β1 attenuated the excitability of noradrenergic neurons of the LC

Figure 3 illustrates the effect of intranasal TGF-β1 administration on the excitability of noradrenergic neurons of the LC. Representative extracellular single-unit recordings are shown in panels A (vehicle) and B (TGF-β1). With respect to the mean neuronal firing rate (C), TGF-β1 significantly decreased firing rate compared to vehicle-treated animals ($p = 0.002$, two-tailed Student's t-test). A similar effect was observed in burst frequency (D), where TGF-β1-treated rats exhibited a significant reduction compared to controls ($p = 0.04$). Analysis of the percentage of spikes occurring within bursts (E) revealed a significant decrease following TGF-β1 administration ($p = 0.01$). Likewise, the mean number of spikes per burst (F) was significantly reduced in the TGF-β1-treated group compared to vehicle-treated animals ($p = 0.01$). No effect on the mean number of the spontaneously active neurons per electrode track was observed (not shown).

Depletion of noradrenaline using fusaric acid reverses effects of TGF-β1 on noradrenergic neurons of LC

Figure 4 illustrates the effect of intranasal TGF-β1 administration on the excitability of noradrenergic neurons of the locus coeruleus (LC) following fusaric acid pretreatment. Representative extracellular single-unit recordings are shown in panels A (vehicle) and B (TGF-β1). With respect to the mean neuronal firing rate (C), no statistically significant difference was observed between vehicle- and TGF-β1-treated animals. Similarly, burst frequency (D) did not differ significantly between groups. The percentage of spikes occurring within bursts (E) also remained unchanged. Analysis of the mean number of spikes per burst (F) revealed no statistically significant difference between vehicle- and TGF-β1-treated animals. No effect on the mean number of the spontaneously active neurons per electrode track was observed (not shown).

LY2109761 stimulated noradrenergic neurons of the LC

Figure 5 illustrates the effect of intracerebroventricular administration of LY2109761 on the excitability of noradrenergic neurons of the LC. Representative extracellular single-unit recordings are shown in panels A (vehicle) and B (LY2109761). With respect to the mean neuronal firing rate (C), LY2109761 significantly increased the firing rate of LC noradrenergic neurons compared to vehicle-treated animals ($p = 0.04$, two-tailed Student's t-test). In contrast, burst frequency (D) did not differ significantly between vehicle- and LY2109761-treated groups. Similarly, no statistically significant differences were observed in the percentage of spikes occurring within bursts (E). Analysis of the mean number of spikes per burst (F) also revealed no significant differences between groups. No effect on the mean number of the spontaneously active neurons per electrode track was observed (not shown).

Discussion

This study aimed to examine the effect of exogeneous TGF-β1 on the excitability of the central 5-HT, dopaminergic, and noradrenergic neurons. We found that the nasal pre-treatment with TGF-β1 had no effect on 5-HT neurons of the DRN and dopaminergic neurons of the VTA, as measured one-hour thereafter. With respect to noradrenergic neurons of the LC, TGF-β1 decreased their mean firing rate and burst activity. This effect of TGF-β1 was observed in naïve rats, but not in the animals underwent noradrenaline depletion by fusaric acid. The selective inhibitor of the TGF-βR1, LY2109761, stimulated noradrenergic neurons of the LC.

We found that acute intranasal administration of TGF-β1 did not alter the firing activity of mature 5-HT neurons in the DRN. Previous work has shown that TGF-β1 can influence serotonergic phenotype development in embryonic raphe cultures, increasing the number of tryptophan hydroxylase-positive neurons by upregulating serotonergic phenotype markers rather than by promoting survival or proliferation [23]. However, these developmental effects are not directly comparable to the present experimental conditions, in which TGF-β1 was administered acutely to adult animals whose DRN serotonergic system was already fully differentiated. Thus, the absence of changes in DRN firing rate, burst activity, or the number of spontaneously active neurons per electrode track indicates that acute TGF-β1 administration does not measurably affect the electrophysiological activity of mature DRN 5-HT neurons under baseline conditions. It was found that TGF-β1 did not alter the excitability of dopaminergic neurons in the VTA. Previous studies have demonstrated that TGF-β1 promotes the survival of rat embryonic midbrain dopaminergic neurons and protects them against 1-methyl-4-phenylpyridinium (MPP^+) toxicity [23]. These effects were attributed to neurotrophic and neuroprotective mechanisms rather than to increased proliferation or astroglial expansion.

In the study by Xue et al., the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a compound that selectively induces dopaminergic degeneration after metabolic conversion to the mitochondrial toxin MPP^+, was used to model Parkinsonian neurodegeneration. Aquaporin-4 deficiency resulted in a failure to upregulate TGF-β1 in the midbrain following MPTP administration, which was associated with enhanced microglial activation and exacerbated loss of tyrosine hydroxylase-positive (TH^+) neurons in the substantia nigra pars compacta (SNpc). Importantly, stereotactic intrastriatal administration of recombinant TGF-β1 attenuated microglial activation and partially rescued dopaminergic neuron survival [24].

Contrary to earlier reports of neuroprotection, adenoviral overexpression of TGF-β1 in the nigrostriatal system aggravated MPTP-induced dopaminergic degeneration and even reduced TH^+ neuron number in mature mice in the absence of toxin exposure, indicating that elevated TGF-β1 levels may exert context-dependent neurotoxic effects on the mesostriatal pathway [25].

In contrast to these findings observed under inflammatory neurotoxic conditions, our electrophysiological recordings in the VTA showed that TGF-β1 did not significantly modify the firing rate of dopaminergic neurons under baseline conditions. This suggests that TGF-β1 may not directly regulate

intrinsic dopaminergic excitability but rather exerts its primary effects through modulation of neuroinflammatory processes and neuronal survival mechanisms.

Intracerebroventricular administration of TGF-β1 was shown to suppress tyrosine hydroxylase expression in the ventral tegmental area and reduce striatal dopamine levels, leading to fatigue-like and depressive behaviors, indicating that TGF-β1 can also directly impair dopaminergic neurotransmission rather than merely affecting neuronal survival [26].

In contrast, our electrophysiological recordings revealed no significant alteration in the baseline firing rate of VTA dopaminergic neurons, suggesting that TGF-β1 may primarily influence dopamine synthesis or release mechanisms rather than intrinsic pacemaker activity under physiological conditions.

In our experiments TGF-β1 significantly reduced the excitability of noradrenergic neurons of LC. In addition, previous studies have shown that TGF-β1 can increase norepinephrine transporter (NET) expression and enhance norepinephrine transport activity in developing noradrenergic neurons [27]. In the study by Sieber-Blum and Ren, TGF-β1 increased NET mRNA levels after approximately 24 h in culture and enhanced norepinephrine transport activity after several days, indicating a relatively slow transcriptional regulatory process. Therefore, although regulation of NET expression may contribute to long-term modulation of noradrenergic signaling, it is unlikely to account for the rapid suppression of LC neuronal firing observed following acute TGF-β1 administration in the present study. Our interpretations of these findings raised the possibility that TGF-β1 alters noradrenergic activity indirectly by modifying extracellular NA levels or NA reuptake within the LC or its projection fields. An increase in extracellular NA could activate inhibitory autoreceptors, such as α2-adrenergic receptors, which are well known to suppress tonic firing and burst activity of LC neurons. Such autoreceptor-mediated feedback represents a key homeostatic mechanism regulating noradrenergic output during stress and arousal [28].

Other studies have reported that intracerebroventricular (i.c.v.) infusion of TGF-β1 induced rapid and long-lasting (7 days) antidepressant effects in a chronic social defeat stress (CSDS) mouse model [12]. Wei and colleagues later demonstrated that also intranasally administered TGF-β1 produces rapid and sustained antidepressant-like effects in CSDS model, reducing immobility in the tail suspension and forced swim tests and restoring sucrose preference at higher doses [13]. Given the central role of the LC–NA system in stress responsiveness, arousal, and behavioral flexibility, suppression of LC hyperactivity provides a plausible neurophysiological mechanism linking TGF-β1 signaling to antidepressant-like effects. This interpretation should be reconciled with the known ability of several noradrenergic antidepressants, including SNRIs, tricyclic antidepressants, and mirtazapine, to enhance noradrenergic neurotransmission. Increased noradrenergic transmission and reduced LC neuronal firing are not necessarily contradictory, because elevated extracellular noradrenaline can activate somatodendritic α2-adrenoceptor-mediated negative feedback in the LC and thereby suppress neuronal firing. Therefore, the TGF-β1-induced reduction in LC firing should not be interpreted simply as reduced noradrenergic neurotransmission. Instead, it may reflect engagement of noradrenaline-dependent feedback mechanisms that restrain LC output, potentially contributing to normalization of stress-related

LC hyperactivity. Further studies measuring extracellular noradrenaline in LC projection areas will be required to test this possibility directly.

In the present study, depletion of noradrenaline using fusaric acid abolished the inhibitory effects of TGF-β1 on the firing activity of locus coeruleus neurons. Fusaric acid is an inhibitor of dopamine-hydroxylase, the enzyme responsible for the conversion of dopamine to noradrenaline and has been previously used experimentally to reduce central noradrenaline levels [18].

By inhibiting this enzyme, fusaric acid leads to decreased synthesis of noradrenaline in the brain and has therefore been employed as a pharmacological tool to study the functional role of noradrenergic systems. An important consideration is that the baseline electrophysiological parameters of LC neurons differed between experimental conditions. In animals pretreated with fusaric acid and receiving intranasal saline, the mean LC firing rate and the mean number of spikes per burst were lower (Fig. 4C,F) than those observed in naïve saline-treated animals (Fig. 3C,F). At first glance, this may appear counterintuitive, because depletion of noradrenaline could be expected to reduce endogenous activation of inhibitory α2-adrenoceptors and thereby facilitate LC neuronal firing. However, fusaric acid inhibits dopamine-β-hydroxylase and therefore interferes with noradrenaline synthesis itself, rather than selectively blocking α2-adrenoceptor-mediated feedback. Thus, prolonged reduction of noradrenaline synthesis may alter LC neuronal activity through mechanisms beyond acute autoreceptor disinhibition, including reduced noradrenergic neurotransmission, altered vesicular transmitter availability, compensatory changes in LC network regulation, or indirect effects on afferent inputs controlling LC excitability. Therefore, the fusaric acid experiment should be interpreted as showing that intact noradrenergic neurotransmission is required for TGF-β1-induced inhibition (Fig. 4C–F), rather than as direct evidence for a simple reduction of α2-autoreceptor tone. To our knowledge, interactions between TGF-β1 and pharmacological depletion of noradrenaline using fusaric acid have not previously been examined using electrophysiological recordings of LC neurons, indicating that the present findings represent a novel observation. The observation that LC activity in fusaric acid-pretreated vehicle animals (Fig. 4C,F) was close to that observed in naïve TGF-β1-treated animals (Fig. 3C,F) suggests that both manipulations may converge on a reduced-output state of the LC–noradrenergic system. However, the mechanisms are likely different: TGF-β1 may acutely enhance inhibitory noradrenergic feedback, whereas fusaric acid reduces noradrenaline synthesis and may thereby disturb normal LC autoregulatory and network function. This distinction is important, because the absence of an additional TGF-β1 effect after fusaric acid pretreatment (Fig. 4C–F) may reflect either occlusion at a low-excitability state or the requirement for intact noradrenergic signaling.

In the present study, blockade of TGF-βR1 receptors with the selective antagonist LY2109761 significantly increased the firing rate of noradrenergic neurons in the LC. To our knowledge, the effects of LY2109761 on the electrophysiological activity of LC neurons have not previously been examined. Therefore, these findings provide the first direct evidence that inhibition of TGF-βR1 signaling can modulate the activity of central noradrenergic neurons. The locus coeruleus represents the principal source of noradrenaline in the brain and plays a critical role in the regulation of arousal, stress

responses, attention, and mood. The increased firing activity observed following LY2109761 administration suggests that endogenous TGF-βR1 signaling may exert a tonic inhibitory influence on LC neuronal activity. Blocking TGF-β receptors could therefore relieve this inhibitory modulation, resulting in enhanced noradrenergic neuronal firing. The lower baseline values observed in the i.c.v. vehicle group used for the LY2109761 experiment (Fig. 5C,F) compared with the intranasal saline vehicle group used for the TGF-β1 experiment (Fig. 3C,F) most likely reflect differences in experimental conditions rather than biological equivalence between the two control groups. Unlike intranasal saline administration (Fig. 3), the i.c.v. vehicle procedure (Fig. 5) involved stereotaxic surgery, penetration of the lateral ventricle, infusion of 5 µL vehicle, and exposure to a vehicle containing DMSO, PEG-300, Tween-80, and water. These factors may influence LC activity through procedural stress, local pressure/volume effects, altered cerebrospinal fluid dynamics, or nonspecific vehicle effects. Therefore, the LY2109761 data were interpreted only relative to their matched i.c.v. vehicle control group (Fig. 5C–F), and not by comparison with the intranasal saline control group (Fig. 3C–F).

Our findings provide the first electrophysiological evidence of TGF-β1's impact on central monoaminergic neurons. Intranasal administration of TGF-β1 reduced excitability of LC noradrenergic neurons, while leaving dopaminergic neurons of the VTA and serotonergic neurons of the DRN unaffected. Early interpretations of these findings raised the possibility that TGF-β1 alters noradrenergic activity indirectly by modifying extracellular NA levels or NA reuptake within the LC or its projection fields.

Depletion of central NA using fusaric acid abolished the inhibitory effects of intranasal TGF-β1 on LC neurons, indicating that intact noradrenergic signaling is required for TGF-β1 to influence LC excitability. This finding supports a mechanism involving noradrenaline-dependent feedback regulation.

Furthermore, intracerebroventricular administration of the TGF-β1 receptor type I antagonist LY2109761 increased NA firing activity, indicating that endogenous TGF-β1 signaling exerts a tonic inhibitory influence on noradrenergic neurons.

In summary, the present data, together with previous behavioral, molecular, and developmental findings, support a model in which TGF-β1 × TGF-βR1 complex might function as a key modulator of noradrenergic system activity. By restraining LC excitability through noradrenaline-dependent mechanisms, TGF-β1 × TGF-βR1 complex may contribute to stress resilience and mood regulation.

Several limitations of the present study should however be acknowledged. First, the experiments were performed under acute conditions in anesthetized animals, which may influence the baseline activity of monoaminergic neurons and potentially modulate the magnitude of pharmacological effects. Second, *in-vivo* single-unit recordings do not provide direct information on synaptic mechanisms or changes in neurotransmitter release, limiting mechanistic interpretation at the cellular and network level. Third, noradrenaline levels were not directly measured in the locus coeruleus or projection areas; therefore, conclusions regarding noradrenaline-dependent feedback mechanisms remain indirect. Fourth, intranasal administration does not allow precise control over the exact brain concentration or regional

distribution of TGF-β1, which may introduce variability in central exposure. Finally, the study focused on acute electrophysiological effects and does not address potential long-term adaptations of monoaminergic systems following repeated TGF-β1 exposure.

Declarations

Conflicts of interest

The authors have no financial interest in publishing this study.

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Author Contribution

Matej Racicky: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing—original draft, Writing—review & editing; Ruslan Paliokha: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing—review & editing: Daniil Grinchii: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing—review & editing; Eliyahu Dremencov Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on request.

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Figures

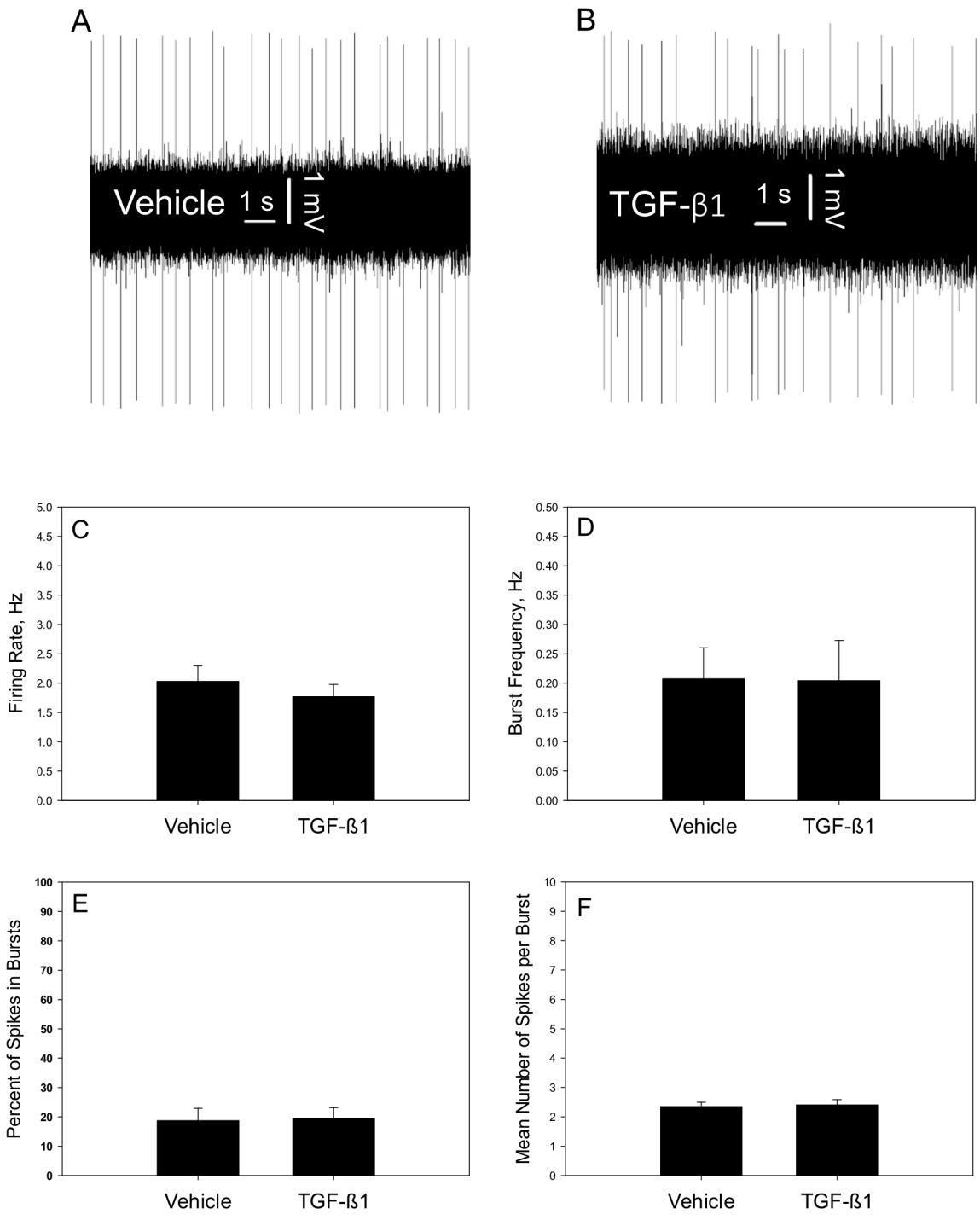


Figure 1

Effect of TGF-β1 on the neuronal parameters of 5-HT neurons in the DRN. Representative extracellular single-unit recordings from a 5-HT neuron following intranasal administration of saline (A) or TGF-β1 (B). Quantitative analysis shows no effect of TGF-β1 on mean firing rate (C), burst frequency (D), percentage of spikes occurring in bursts (E), and mean number of spikes per burst (F). Data from 39 neurons from 5 saline-treated rats and 42 neurons from 6 TGF-β1-treated rats.

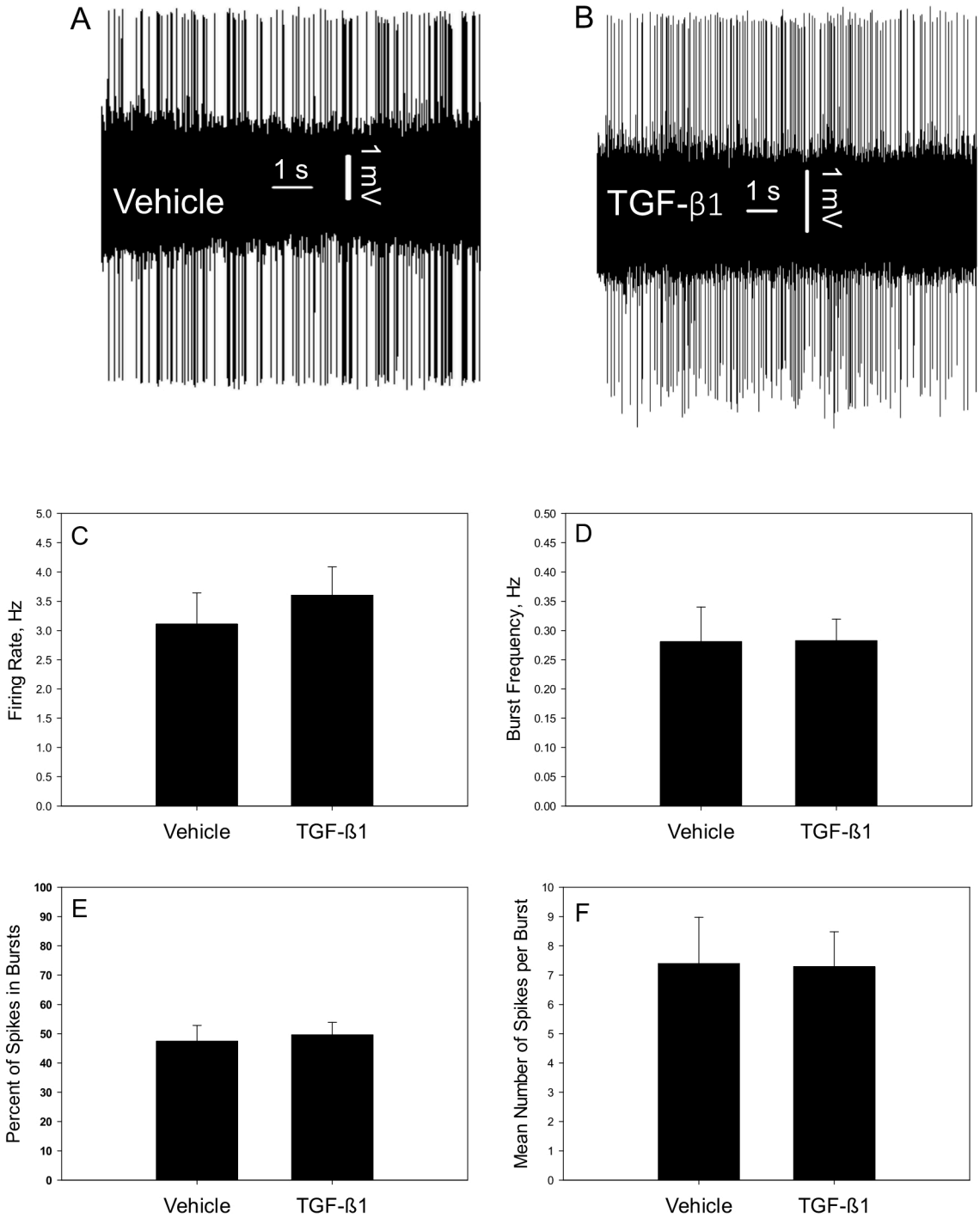


Figure 2

Effect of TGF-β1 on the neuronal parameters of dopaminergic neurons in the VTA. Representative extracellular single-unit recordings from a dopaminergic neuron following intranasal administration of saline (A) or TGF-β1 (B). Scale bars indicate 1 s and 1 mV. Quantitative analysis shows no effect of TGF-β1 on mean firing rate (C), burst frequency (D), percentage of spikes occurring in bursts (E), and mean

number of spikes per burst (F). Data were calculated from 49 neurons from 5 saline-treated rats and 47 neurons from 6 TGF-β1-treated rats.

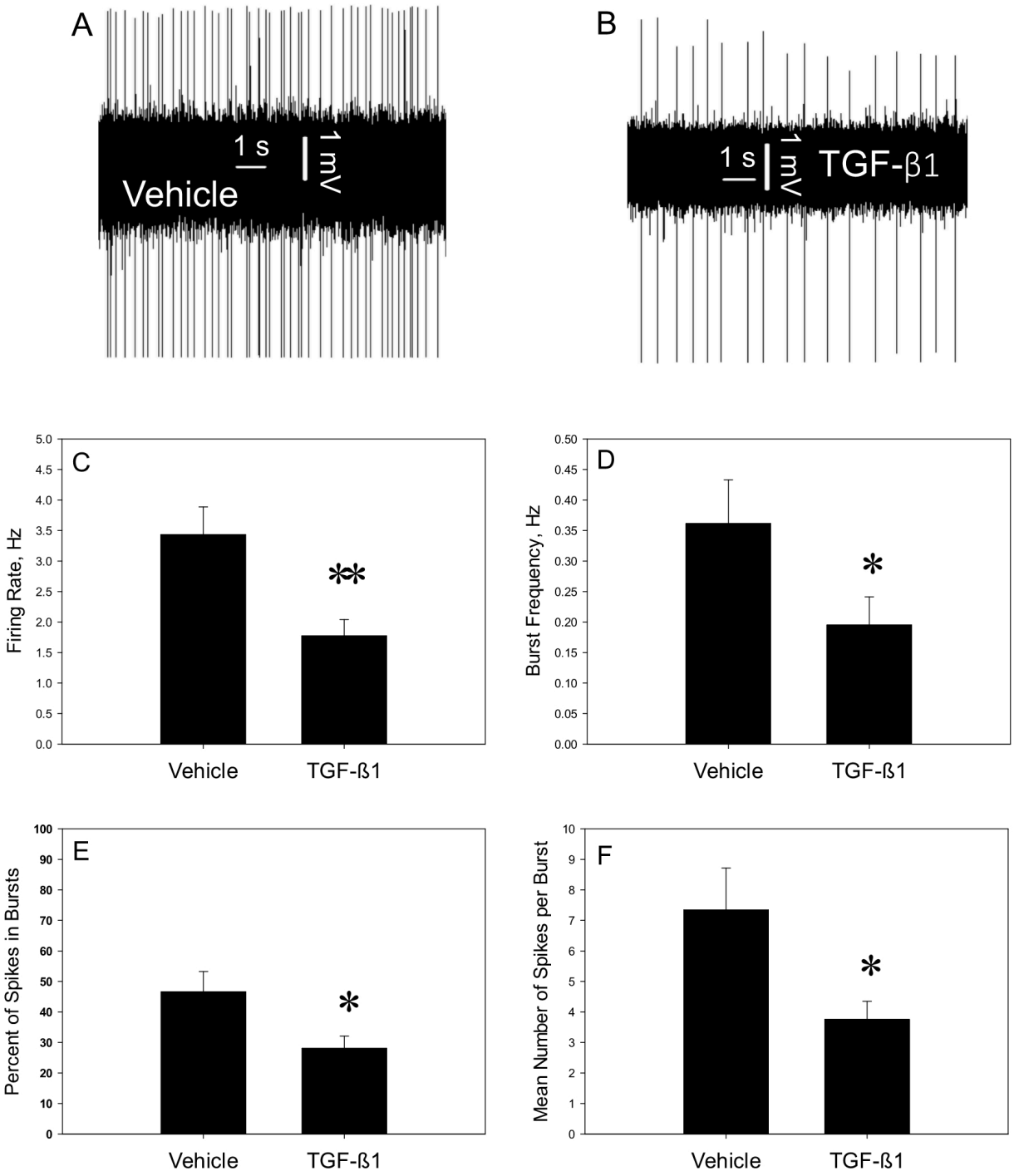


Figure 3

Effect of TGF-β1 on the neuronal parameters of noradrenergic neurons in the LC. Representative extracellular single-unit recordings from a noradrenergic neuron following intranasal administration of

saline (A) or TGF-β1 (B)[20]. Scale bars indicate 1 s and 1 mV. Quantitative analysis shows the effect of TGF-β1 on mean firing rate (C), burst frequency (D), percentage of spikes occurring in bursts (E), and mean number of spikes per burst (F). Data were calculated from 21 neurons from 4 saline-treated rats and 30 neurons from 5 TGF-β1-treated rats. Statistical significance is indicated by *p<0.05 and **p<0.01 compared to vehicle-treated controls (two-tailed Student's t-test).

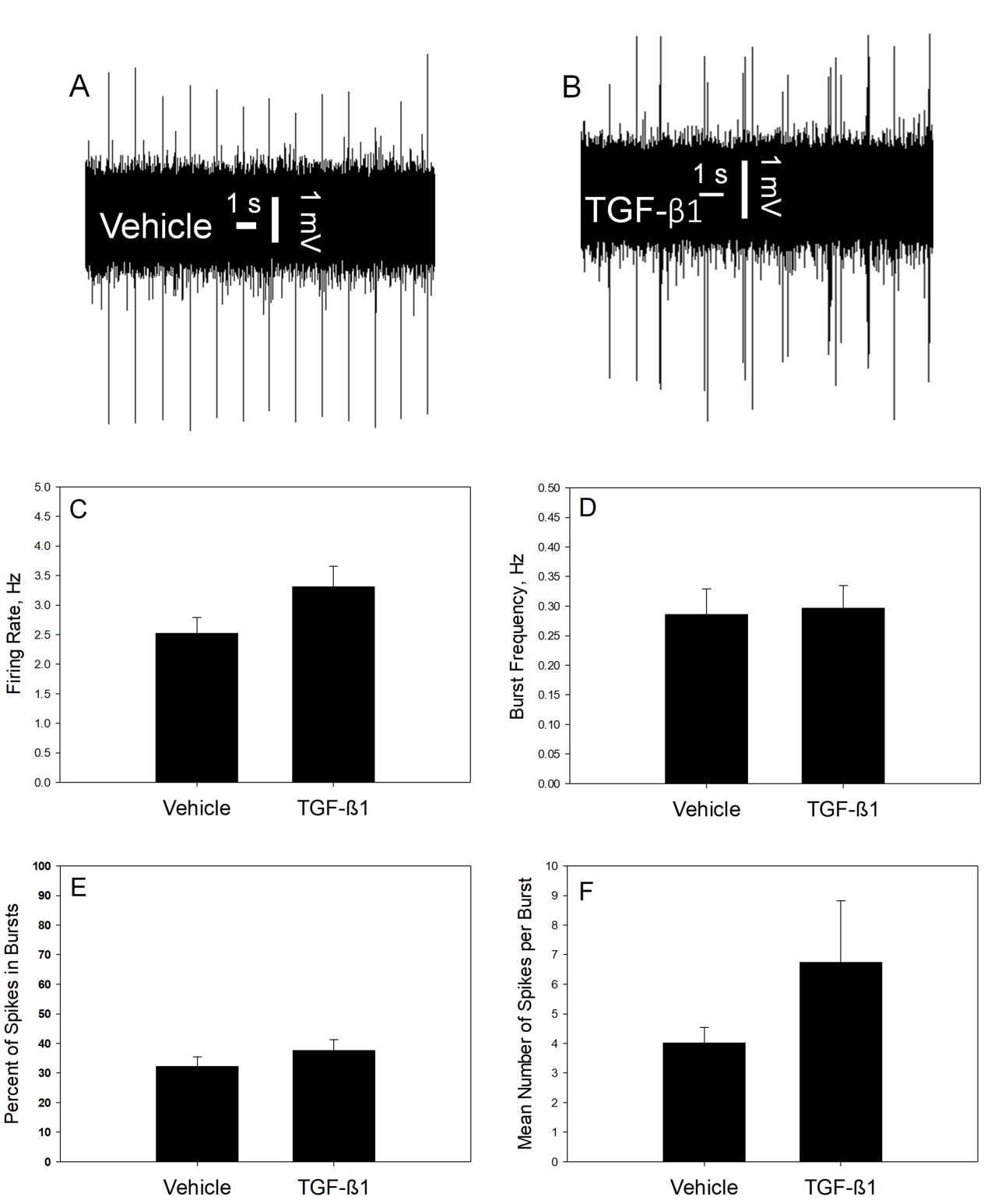


Figure 4

Effect of TGF-β1 on the neuronal parameters of noradrenergic neurons in the LC after pre-treatment with fusaric acid. Representative extracellular single-unit recordings from a noradrenergic neuron after pre-treatment with fusaric acid, following intranasal administration of saline (A) or TGF-β1 (B). Scale bars indicate 1 s and 1 mV. Quantitative analysis shows the effect of fusaric acid treatment in combination with saline or TGF-β1 on mean firing rate (C), burst frequency (D), percentage of spikes occurring in bursts (E), and mean number of spikes per burst (F). Data were calculated from 51 neurons from 5 saline-treated rats and 62 neurons from 5 TGF-β1-treated rats. No statistically significant differences were observed between groups (two-tailed Student's t-test).

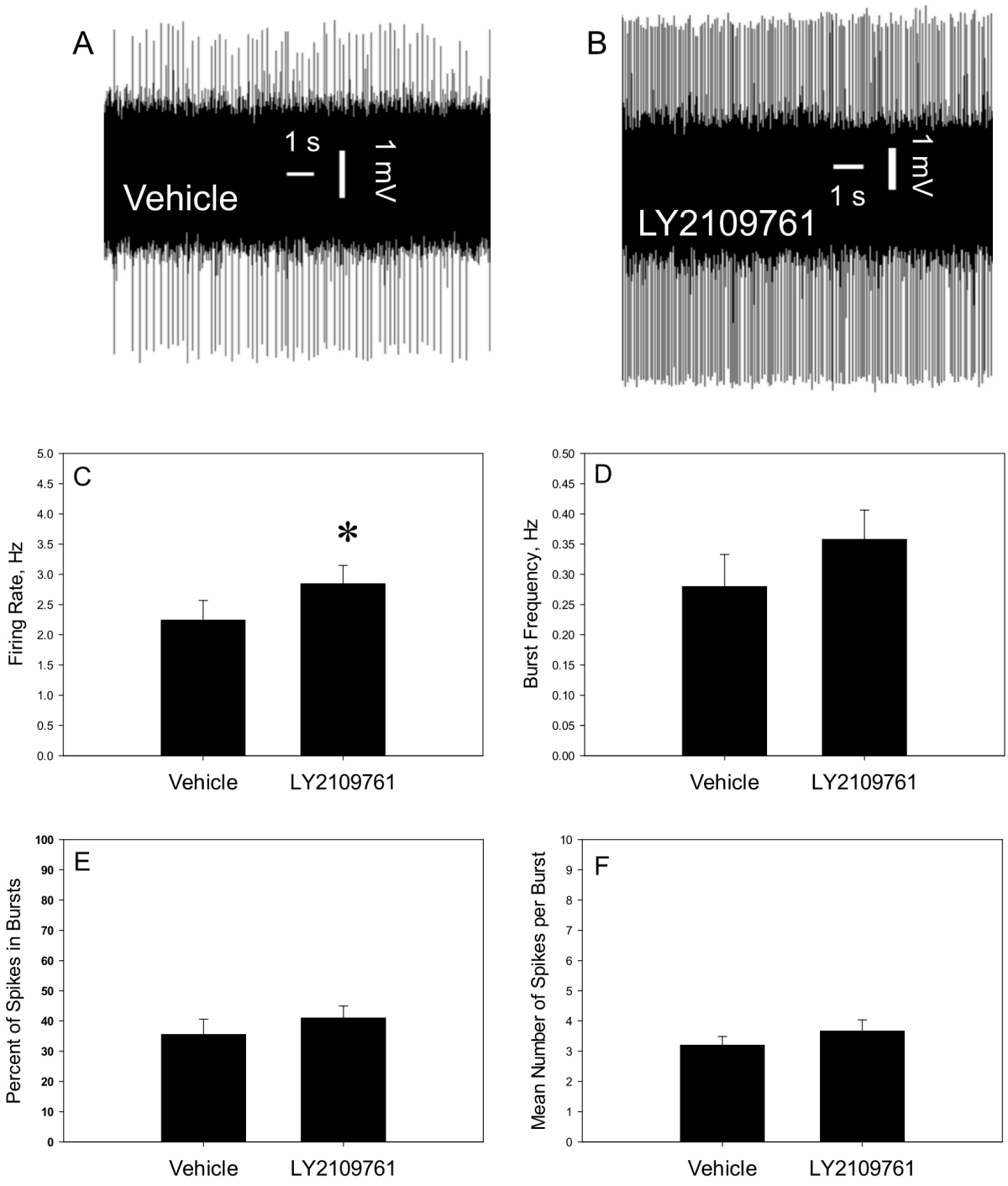


Figure 5

Effect of LY2109761 on the neuronal parameters of noradrenergic neurons in the LC. Representative extracellular single-unit recordings from a noradrenergic neuron in the LC following intracerebroventricular administration of vehicle (A) or LY2109761 (B). Scale bars indicate 1 s and 1 mV. Quantitative analysis shows the effect of LY2109761 on mean firing rate (C), burst frequency (D), percentage of spikes occurring in bursts (E), and mean number of spikes per burst (F). Data were

calculated from 21 neurons from 5 vehicle-treated rats and 34 neurons from 5 LY2109761-treated rats. Statistical significance is indicated by *$p<0.05$ compared to vehicle-treated controls (two-tailed Student's t-test).