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Original Article

Pain regulation by oxytocin in an adult rat model of neuralgia

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Abstract: Neuralgia is a disorder characterized by persistent chronic pain, and clinical treatment regimens for neuralgia are often unsatisfactory. The present study used an adult rat model of neuralgia to examine the of oxytocin (OT) microinjected into the dorsal raphe nucleus on pain regulation. Hind paw withdrawal latencies (HWLs) to thermal stimulation (using a hot plate) and mechanical stimulation (using a pressure plate) were measured as an indicator of pain 5, 10, 15, 20, 30, 45 and 60 min after microinjections of OT (1.25, 2.5, or 5.0 nmol/μL) or saline (control). Compared with saline, OT significantly prolonged HWL. Rats injected with OT at a concentration of 1.25 nmol/μL displayed a significant difference in HWL to thermal stimulation for the left ($P < 0.001$) and right ($P < 0.001$) hind paws and also displayed a significant difference in HWL to mechanical stimulation for the left ($P < 0.001$) and right ($P < 0.05$) hind paws. Rats injected with OT concentrations of 2.5 and 5.0 nmol/μL displayed a significant difference ($P < 0.001$) in HWL to thermal or mechanical stimulation between the left and right hind paws, with the difference most apparent 15 min after the injection. These data indicate that OT microinjected into the dorsal raphe nucleus can prolong HWL in adult rats modeling neuralgia and that the effect of OT is dose dependent, suggesting that OT in the dorsal raphe nucleus may promote analgesia in a rat model of neuralgia.

Keywords: Oxytocin, dorsal raphe nucleus, microinjection, HWL, analgesia

Introduction

Chronic pain is a common disorder for which there are few successful treatment methods. Such pain is primarily caused by inflammation and nerve injury and lasts for a long time [1]. However, owing to the lack of an appropriate animal model of chronic pain, there is a dearth in both the understanding of long-term pain etiologies and the development of novel therapeutics [2]. Neuralgia is a disorder characterized by persistent chronic pain with uncertain etiology; thus, clinical treatment regimens are often unsatisfactory. In the present study, an adult rat model of neuralgia was established to examine the analgesic effects of oxytocin (OT) in this disorder.

A neurohypophysial hormone generated by the giant cells of the supraoptic nucleus and the paraventricular nucleus in the hypothalamus, OT is transported by the hypothalamus-pituitary axis nerve fibers to the posterior pituitary for secretion and release into the blood [3]. OT is composed of nine amino acid residues, of which six amino acids form a ring at the C-terminus and three amino acids form the end of the N-terminus [4]. In the central nervous system, OT expression is mainly present in the hypothalamus and supraoptic nucleus [5]. Cells containing OT include large cells with their axons terminating at the neurohypophysis and small neural cells with their axons terminating at other points in the central nervous system. Fibers and terminals containing OT can be detected in various cerebral areas [6, 7]. Owing to its actions on uterine smooth muscle, OT can enhance uterine contractions but does not generate a substantial effect during the early or middle stage of pregnancy. However, the uterus becomes more sensitive to OT during the late stage of pregnancy, at which time the effects of OT can be readily observed [8]. OT can also gen-

erate an extensive range of physiological effects, including effects on the mammary and pituitary glands [9], regulation of cardiovascular function, influences on gastric motility and secretion functions, enhancement of degenerative amnesia, and participation in pain regulation [10, 11].

Located on the ventral aspect of the aqueduct of the midbrain, the dorsal raphe nucleus (DRN) serves as an important nuclear cluster in human pain regulation [12, 13]. The DRN has nerve fiber connections with many brain regions participating in pain regulation, including the periaqueductal gray and locus coeruleus [14]. Therefore, research assessing the analgesic effect of OT and its mechanisms of action are needed, and the results of such studies may reveal novel relationships among nuclear clusters relevant to the analgesic effect of OT and its regulation. However, currently no studies published in China have investigated the influence of OT in the DRN on pain regulation in the adult rat. Thus, in the present study, an adult rat model of neuralgia was established. Noxious thermal stimulation (hot plate) or mechanical stimulation (pressure plate) was applied to the hind paw of the rats, and hind paw withdrawal latency (HWL) was measured as an indicator of pain. The pain regulating effects of OT microinjections into the DRN were thus determined to assess the analgesic effect of OT and its regulation to provide a basis for the clinical application of an OT receptor antagonist.

Materials and methods

Experimental animals

Adult male Wistar rats (n = 14; weight, 180-220 g) were provided by the Animal Center of the College of Basic Medical Sciences at Jilin University. During the experiments, the rats were kept in separate cages and fed standard rat chow and water ad libitum. The temperature was maintained between 16 and 24°C, and a 12 h light/dark cycle was used.

Drugs and reagents

The following drugs and reagents were used: oxytocin (Chinapeptides Co., Ltd., Shanghai, China) at concentrations of 1.25, 2.5 and 5.0 nmol/μL prepared with saline; 10% chloral hydrate; medicinal alcohol; 30% hydrogen peroxide; methylene blue; and acrylic denture base resin powder and liquid.

Instruments

The intelligent hot plate apparatus (YLS-6B) and electronic pressure algometer (LYS-3E) were both manufactured by Anhui Zhenghua Biological Instruments & Equipment Co., Ltd. The stereotaxic apparatus was manufactured by RWD Life Science. Other instruments used included surgical instruments, syringes of different sizes, an electronic balance, dropper, large and small beakers, a manual drill, stainless steel trocars, microsyringes, and polyethylene tubing.

Establishment of the adult rat model of neuralgia

Adult rats were anesthetized using an intraperitoneal injection of 10% chloral hydrate and placed in a lateral position. The hair was removed from the left femur with scissors, and the skin was disinfected with iodine tincture and swabbed with alcohol. The skin was incised approximately 0.5 cm below a line connecting the hip and knee joint. The sciatic nerve was exposed by separating the muscles and ligated four times at intervals of 1 mm with #1 suture. The incision was sutured layer by layer and sprinkled with penicillin powder to prevent infection.

Experimental groups and drug administration

Rats were divided into the following four groups: OT control (1.25, 2.5 and 5.0 nmol/μL OT); saline control; OT neuralgia (1.25, 2.5 and 5.0 nmol/μL OT); and saline neuralgia groups. All rats in each group were anesthetized with an intraperitoneal injection of 10% chloral hydrate and then fixed in a stereotaxic apparatus with the head held horizontally. The skull was exposed and the location of the DRN was determined using Paxinos and Watson's atlas *The Rat Brain in Stereotaxic Coordinates*. A trocar was implanted in the DRN of rats and fixed with acrylic resin base denture powder and liquid, and the gap between the trocar and the skull was also sealed with the acrylic resin based denture powder and liquid. Two and 3 days after surgery, behavioral pain testing was performed and HWL was measured. Rats in both the control and neuralgia model groups were

Table 1. HWL to Thermal or Mechanical Stimulation in Normal Rats after microinjection of OT at a Concentration of 1.25 nmol/μL

Handling method		Side	HWL (s)	The percent change of HWL after trace injection in DRN of rat							F value	*P* value
				5 min	10 min	15 min	20 min	30 min	45 min	60 min		
Hot plate test	OT	L	193.76 ± 1.96	9.87 ± 3.17	14.75 ± 1.81	17.60 ± 1.89	16.14 ± 1.80	13.46 ± 1.36	11.34 ± 2.53	5.27 ± 2.47	32.36	0.00005***
		R	188.2 ± 1.34	11.92 ± 2.54	14.38 ± 2.22	18.56 ± 1.24	16.93 ± 1.94	14.69 ± 1.21	12.53 ± 2.52	9.13 ± 2.07	45.03	0.000009***
	Saline	L	194.31 ± 1.53	-0.88 ± 2.05	-1.91 ± 2.40	-1.71 ± 1.59	-1.64 ± 2.69	0.91 ± 2.00	-1.58 ± 2.54	-1.34 ± 1.36		
		R	194.21 ± 2.70	-5.88 ± 1.57	0.02 ± 2.21	-3.58 ± 1.57	-1.93 ± 1.97	-2.05 ± 1.97	-3.53 ± 2.71	-0.84 ± 2.40		
Pressure plate test	OT	L	12091.82 ± 3645.82	10.45 ± 2.60	16.03 ± 2.83	20.70 ± 1.58	16.41 ± 2.48	12.73 ± 1.91	7.19 ± 2.41	1.41 ± 2.53	12.52	0.003**
		R	11686.06 ± 104.86	14.59 ± 2.16	18.97 ± 2.52	22.71 ± 2.14	16.72 ± 3.22	15.28 ± 2.88	11.76 ± 1.64	3.26 ± 0.72	22.17	0.0003***
	Saline	L	12238.10 ± 3270.77	0.28 ± 3.21	2.17 ± 2.53	-1.64 ± 2.37	0.98 ± 2.29	1.50 ± 2.38	4.40 ± 2.45	1.58 ± 2.29		
		R	12234.05 ± 129.50	-0.29 ± 2.46	-2.19 ± 1.50	-0.57 ± 3.74	3.64 ± 1.27	2.46 ± 1.66	-4.43 ± 2.64	2.18 ± 2.72		

Abbreviations: DRN, dorsal raphe nucleus; HWL, hind paw withdrawal latency; L, left; OT, oxytocin; R, right. Control group microinjected with 5 μL of 0.9% saline. Comparisons among data at various time points in the same group were conducted with two-way repeated measures ANOVA. Comparisons of differences between groups were conducted using two-tailed *t* tests. Comparison between left and right sides was conducted with paired *t* tests. Data expressed as mean ± S.E.M. **P < 0.01 and ***P < 0.001 compared with the control group at the corresponding time point.

Table 2. HWL to Thermal or Mechanical Stimulation in Normal Rats after microinjection of OT at a Concentration of 2.5 nmol/μL

Handling method		Side	HWL (s)	The percent change of HWL after trace injection in DRN of rats							F value	*P* value
				5 min	10 min	15 min	20 min	30 min	45 min	60 min		
Hot plate test	OT	L	190.36 ± 2.15	16.65 ± 3.06	24.67 ± 3.50	27.81 ± 3.32	23.78 ± 2.68	16.22 ± 2.53	15.21 ± 1.62	10.31 ± 2.09	31.53	0.00006***
		R	190.07 ± 1.28	15.71 ± 3.54	23.93 ± 3.16	25.90 ± 4.71	22.85 ± 2.78	15.314 ± 2.92	12.55 ± 2.33	6.83 ± 2.77	29.62	0.00008***
	Saline	L	194.31 ± 1.53	-0.88 ± 2.05	-1.91 ± 2.40	-1.71 ± 1.59	-1.64 ± 2.69	0.91 ± 2.00	-1.58 ± 2.54	-1.34 ± 1.36		
		R	194.21 ± 2.70	-5.88 ± 1.57	0.02 ± 2.21	-3.58 ± 1.57	-1.93 ± 1.97	-2.05 ± 1.97	-3.53 ± 2.71	-0.84 ± 2.40		
Pressure plate test	OT	L	12390.24 ± 3311.43	9.73 ± 1.85	18.56 ± 2.50	22.79 ± 2.28	18.20 ± 2.74	14.18 ± 1.64	11.78 ± 2.65	9.97 ± 1.03	21.94	0.0003***
		R	11882.38 ± 201.73	19.90 ± 3.33	22.99 ± 2.19	24.58 ± 2.95	20.04 ± 3.11	18.19 ± 2.66	13.52 ± 1.75	10.50 ± 2.13	32.51	0.00005***
	Saline	L	12238.10 ± 3270.77	0.28 ± 3.21	2.17 ± 2.53	-1.64 ± 2.37	0.98 ± 2.29	1.50 ± 2.38	4.40 ± 2.45	1.58 ± 2.29		
		R	12234.05 ± 129.50	-0.29 ± 2.46	-2.19 ± 1.50	-0.57 ± 3.74	3.64 ± 1.27	2.46 ± 1.66	-4.43 ± 2.64	2.18 ± 2.72		

Abbreviations: DRN, dorsal raphe nucleus; HWL, hind paw withdrawal latency; L, left; OT, oxytocin; R, right. Control group microinjected with 5 μL of 0.9% saline. Comparisons among data at various time points in the same group were conducted with two-way repeated measures ANOVA. Comparisons of differences between groups were conducted using two-tailed *t* tests. Comparisons between left and right sides were conducted with paired *t* tests. Data expressed as mean ± S.E.M. ***P < 0.001 compared with the control group at the corresponding time point.

Table 3. HWL to Thermal or Mechanical Stimulation in Normal Rats after microinjection of OT at a Concentration of 5.0 nmol/μL

Handling method		Side	HWL (s)	The percent change of HWL after trace injection in DRN of rats							F value	*P* value
				5 min	10 min	15 min	20 min	30 min	45 min	60 min		
Hot plate test	OT	L	189.54 ± 2.40	20.10 ± 2.72	26.68 ± 3.52	31.41 ± 3.11	25.97 ± 2.43	19.99 ± 1.52	16.22 ± 2.57	10.72 ± 2.68	31.36	0.00006***
		R	189.33 ± 1.96	19.30 ± 2.20	27.52 ± 2.46	32.12 ± 3.28	23.90 ± 1.80	19.44 ± 1.98	14.84 ± 3.27	12.77 ± 3.35	34.43	0.00004***
	Saline	L	194.31 ± 1.53	-0.88 ± 2.05	-1.91 ± 2.40	-1.71 ± 1.59	-1.64 ± 2.69	0.91 ± 2.00	-1.58 ± 2.54	-1.34 ± 1.36		
		R	194.21 ± 2.70	-5.88 ± 1.57	0.02 ± 2.21	-3.58 ± 1.57	-1.93 ± 1.97	-2.05 ± 1.97	-3.53 ± 2.71	-0.84 ± 2.40		
Pressure plate test	OT	L	12408.21 ± 3441.42	16.52 ± 3.04	22.97 ± 1.93	28.22 ± 4.11	22.11 ± 2.78	16.93 ± 1.75	13.02 ± 2.45	8.89 ± 1.70	21.74	0.0003***
		R	11915.13 ± 144.34	16.70 ± 4.24	30.57 ± 3.56	35.04 ± 3.55	25.58 ± 1.89	20.57 ± 2.57	15.96 ± 3.26	13.96 ± 2.55	27.13	0.0001***
	Saline	L	12238.10 ± 3270.77	0.28 ± 3.21	2.17 ± 2.53	-1.64 ± 2.37	0.98 ± 2.29	1.50 ± 2.38	4.40 ± 2.45	1.58 ± 2.29		
		R	12234.05 ± 129.50	-0.29 ± 2.46	-2.19 ± 1.50	-0.57 ± 3.74	3.64 ± 1.27	2.46 ± 1.66	-4.43 ± 2.64	2.18 ± 2.72		

Abbreviations: DRN, dorsal raphe nucleus; HWL, hind paw withdrawal latency; L, left; OT, oxytocin; R, right. Control group microinjected with 5 μL of 0.9% saline. Comparisons among data at various time points in the same group were conducted with two-way repeated measures ANOVA. Comparisons of differences between groups were conducted using two-tailed *t* tests. Comparisons between left and right sides were conducted with paired *t* tests. Data expressed as mean ± S.E.M. ***P < 0.001 compared with the control group at the corresponding time point.

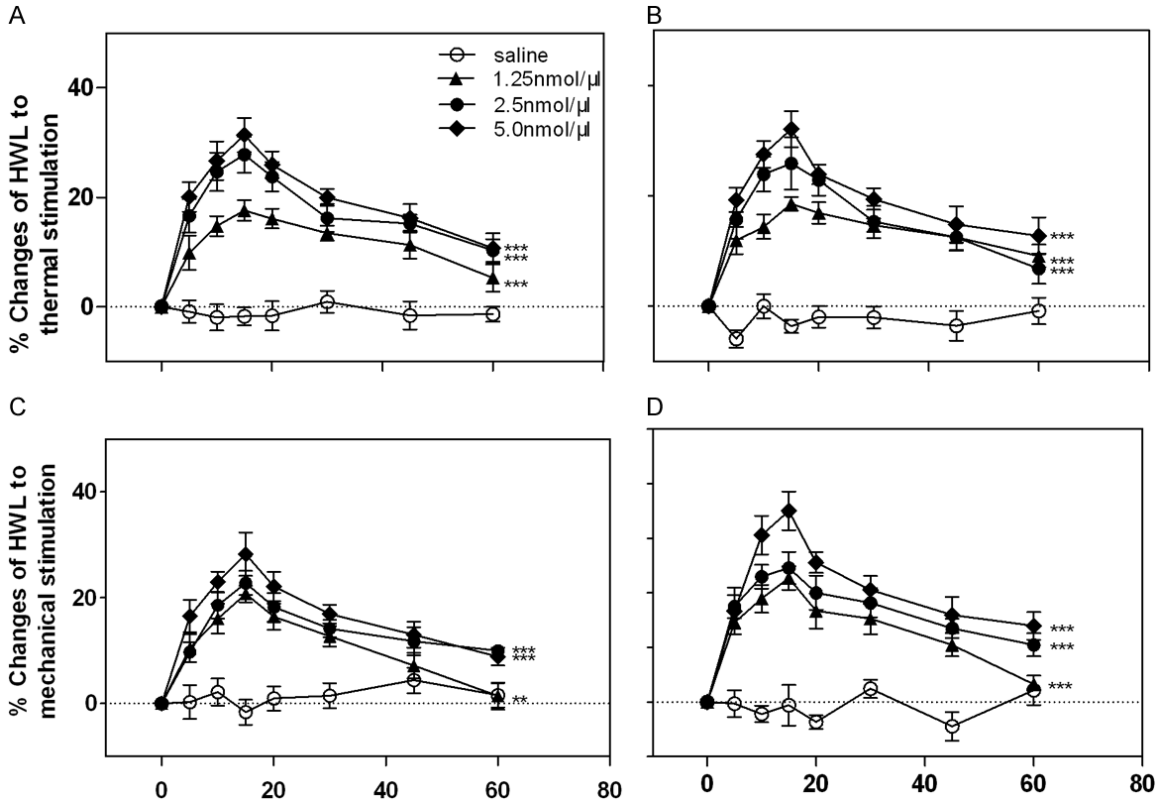

Figure 1. Percentage of HWL change in normal adult rats injected with saline or various concentrations of oxytocin (OT). Differences between groups were analyzed with one-way ANOVA. Pairwise data comparisons were conducted with least significant difference or Student-Newman-Keuls post hoc tests. *P < 0.05 vs. normal group. Effects of DRN OT microinjections at concentrations of 1.25, 2.5, or 5.0 nmol/μL on the HWLs to thermal (A and B) and mechanical (C and D) stimulation in normal rats. Control rats received a DRN microinjection of 1 μL of 0.9% saline. HWL indicates hind paw withdrawal latency. Data are presented as means ± S.E.M. The statistical significance of the differences between the groups was determined using one-way ANOVA.

injected with 1.25, 2.5, or 5.0 nmol/μL OT or saline through the inserted trocar. For each injection, the syringe remained in the trocar for 30 s after the injection before being removed.

Detection of HWL

At 5, 10, 15, 20, 30, 45 and 60 min after the rats in the control and neuralgia groups were injected with the various concentrations of OT or saline, a hot plate test and a pressure test was performed to determine their HWLs. To familiarize the rats with the experimental environment and to reduce experimental errors, all rats were subjected to both the hot plate and pressure tests before injection of OT or saline. On the basis of the experimental method proposed by Sun et al. [15], the tests were conducted each day for 5 days, with three hot plate and three pressure tests per day. The HWL before OT or saline injection was measured three times, and the means of these three values for each test were set as baseline values [16]. The HWLs of rats in each group at different time points after OT or saline injections of drugs were measured and expressed as percentage changes from the baseline value calculated according to the following formula: HWL% = [(HWL of rat after injection - HWL baseline value before injection)/HWL baseline value before injection] × 100%.

Hot plate test

A hot plate was used to measure the reaction of the rats to noxious thermal stimulation. The temperature of the hot plate was maintained at approximately 52°C. The rat was held by the experimenter with one hand, and its hind limbs were controlled with the experimenter's other hand so that the hind paw fully contact the surface of the hot plate. The time measurement began when the rat's paw touched the plate and stopped when the rat voluntarily withdrew

its paw from the surface of the hot plate. This measurement served as the HWL for noxious thermal stimulation.

Pressure test

Pressure pain detection threshold was measured using a pressure algometer to determine the reaction of the rat to noxious mechanical stimulation. An adult rat was held by the experimenter with one hand and the rat's hind limbs were controlled with the experimenter's other hand. The animal's paw was placed between a small wedge-shaped glass and the surface of the pressure algometer, with the glass pressed against the dorsum of the foot. Progressively increasing pressure was exerted at the constant rate of 30 g/s. When the rat withdrew its hind limb, the pressure was stopped, and the pressure value was read and used as the HWL for mechanical stimulation.

Statistical analysis

All data are expressed as means ± standard error of the mean (S.E.M.). Differences between groups were compared using one-way analysis of variance (ANOVA). Pairwise data comparisons were conducted with least significant difference or Student-Newman-Keuls post hoc tests. Differences between groups were analyzed using paired *t* tests. Values of *P* less than 0.05 were considered to be statistically significant.

Results

After injection of OT into the DRN of normal rats, HWL was prolonged and increased with increasing OT concentration

After DRN microinjection of OT into normal rats at concentrations of 1.25 (**Table 1**), 2.5 (**Table 2**), and 5.0 nmol/μL (**Table 3**; **Figure 1**), there were significant differences in the HWLs of the left and right hind paws ($P < 0.001$) compared with those in the control group that received saline microinjections. After microinjection of OT at a concentration of 1.25 nmol/μL, adult rats displayed a significant difference in HWL of the left hind paw ($P < 0.01$) and a significant difference in the HWL of the right hind paw ($P < 0.001$) compared with those in the control group that received saline microinjections (**Table 1**). After microinjection of OT at concentrations of 2.5 nmol/μL (**Table 2**) and 5 nmol/μL (**Table 3**) into the DRN, adult rats have a significant difference in the HWL of left and right hind paws ($P < 0.001$) compared with those in the control group that received saline microinjections. These results indicate that OT microinjections into the DRN lead to a significant analgesic effect.

After injection of OT into the DRN in adult rats modeling neuralgia, HWL wass prolonged and increased with increasing OT concentration

After DRN microinjections of OT at concentrations of 1.25 (**Table 4**) and 2.5 nmol/μL (**Table 5** and **Figure 2**), adult rats modeling neuralgia showed a significant difference in HWL to thermal stimulation of the left and right hind paws ($P < 0.001$) compared with the control group. After microinjection of OT at a concentration of 5 nmol/μL (**Table 6**), adult rats modeling neuralgia showed a significant difference in the HWL to thermal stimulation of the left hind paws ($P < 0.01$) and a significant difference in the HWL of the right hind paws ($P < 0.001$) compared with those of the control group. After microinjection into the DRN of OT at a concentration of 1.25 nmol/μL (**Table 4**), adult rats modeling neuralgia showed a significant difference in HWL to mechanical stimulation of left hind paws ($P < 0.001$) and a significant difference in the HWL of the right hind paws ($P < 0.05$) compared with the control group. After microinjection into the DRN of OT at concentrations of 2.5 nmol/μL (**Table 5**) and 5 nmol/μL (**Table 6**), adult rats modeling neuralgia displayed a significant difference in HWL to mechanical stimulation of the left and right hind paws ($P < 0.001$) compared with the control group. As shown in **Tables 4**-**6**, microinjection of OT into the DRN of both normal adult rats and an adult rat model of neuralgia generated a significant dose-dependent analgesic effect that was most apparent 15 minutes after the injection.

Under certain conditions, HWL was longer in adult rats modeling neuralgia than that in normal adult rats after microinjection of OT into the DRN

The analgesic effects generated 15 minutes after injection of 5 nmol/μL OT into the DRN were compared between normal adult rats and adult rats modeling neuralgia. Although no sig-

Table 4. HWL to Thermal or Mechanical Stimulation in Adult Rats Modeling Neuralgia after microinjection of OT at a Concentration of 1.25 nmol/μL

Handling method		Side	HWL (s)	The percent change of HWL after trace injection in DRN of rats							F value	*P* value
				5 min	10 min	15 min	20 min	30 min	45 min	60 min		
Hot plate test	OT	L	271.61 ± 8.76	12.29 ± 5.71	10.91 ± 2.53	16.91 ± 3.46	9.49 ± 3.20	3.85 ± 3.14	2.14 ± 3.92	-2.78 ± 3.31	17.66	0.000887***
		R	189.22 ± 5.70	8.33 ± 3.51	11.63 ± 3.72	14.62 ± 2.15	7.44 ± 3.64	6.29 ± 3.03	5.50 ± 3.26	2.15 ± 4.90	35.66	0.000034***
	Saline	L	273.42 ± 35.84	3.48 ± 6.55	-3.07 ± 3.34	-5.39 ± 5.64	-4.22 ± 5.87	8.22 ± 7.13	-1.51 ± 8.55	6.09 ± 4.37		
		R	169.58 ± 9.85	-6.75 ± 6.59	-2.20 ± 10.02	-6.12 ± 5.38	-832. ± 6.37	-2.48 ± 4.77	-9.38 ± 5.05	-5.80 ± 5.40		
Pressure plate test	OT	L	13285.00 ± 5423.58	2.79 ± 1.87	5.93 ± 2.31	8.48 ± 1.36	5.42 ± 2.75	4.83 ± 2.62	2.56 ± 1.34	0.84 ± 2.77	20.97	0.000429***
		R	11074.11 ± 135.90	4.72 ± 3.87	5.76 ± 3.58	8.82 ± 3.36	4.57 ± 3.02	3.57 ± 2.99	2.02 ± 2.41	1.97 ± 1.58	6.15	0.02650*
	Saline	L	14180.83 ± 7.90.42	-0.09 ± 2.36	-7.07 ± 2.83	-6.89 ± 1.82	-2.34 ± 4.93	-7.07 ± 2.93	-4.29 ± 3.51	0.88 ± 2.97		
		R	10.926.67 ± 128.11	-2.19 ± 4.33	2.57 ± 2.39	3.35 ± 1.50	-0.34 ± 2.20	1.52 ± 4.20	1.25 ± 2.09	2.39 ± 2.99		

Abbreviations: DRN, dorsal raphe nucleus; HWL, hind paw withdrawal latency; L, left; OT, oxytocin; R, right. Control group microinjected with 5 μL of 0.9% saline. Comparisons among data at various time points in the same group were conducted with two-way repeated measures ANOVA. Comparisons of differences between groups were conducted using two-tailed *t* tests. Comparisons between left and right sides were conducted with paired *t* tests. Data expressed as mean ± S.E.M. *P < 0.05 and ***P < 0.001 compared with the control group at the corresponding time point.

Table 5. HWL to Thermal or Mechanical Stimulation in Adult Rats Modeling Neuralgia after microinjection of OT at a Concentration of 2.5 nmol/μL

Handling method		Side	HWL (s)	The percent change of HWL after trace injection in DRN of rats							F value	*P* value
				5 min	10 min	15 min	20 min	30 min	45 min	60 min		
Hot plate test	OT	L	314.57 ± 19.53	16.25 ± 8.89	18.94 ± 8.76	22.48 ± 6.81	13.11 ± 5.42	7.25 ± 6.92	6.57 ± 4.32	6.70 ± 5.36	23.87	0.000240***
		R	175.67 ± 2.71	9.17 ± 2.74	15.08 ± 6.33	19.94 ± 2.88	16.49 ± 6.40	12.95 ± 4.22	9.28 ± 2.61	8.40 ± 4.72	45.45	0.00000944***
	Saline	L	273.42 ± 35.84	3.48 ± 6.55	-3.07 ± 3.34	-5.39 ± 5.64	-4.22 ± 5.87	8.22 ± 7.13	-1.51 ± 8.55	6.09 ± 4.37		
		R	169.58 ± 9.85	-6.75 ± 6.59	-2.20 ± 10.02	-6.12 ± 5.38	-832 ± 6.37	-2.48 ± 4.77	-9.38 ± 5.05	-5.80 ± 5.40		
Pressure plate test	OT	L	13793.33 ± 5213.39	8.25 ± 2.11	11.31 ± 3.46	14.49 ± 3.18	7.84 ± 1.69	6.74 ± 0.88	4.53 ± 2.94	1.90 ± 2.01	24.49	0.000214***
		R	11029.52 ± 167.91	6.14 ± 2.34	15.40 ± 1.80	19.05 ± 2.68	15.01 ± 3.30	12.93 ± 4.74	9.56 ± 2.51	5.91 ± 2.98	16.73	0.00110**
	Saline	L	14180.83 ± 7.90.42	-0.09 ± 2.36	-7.07 ± 2.83	-6.89 ± 1.82	-2.34 ± 4.93	-7.07 ± 2.93	-4.29 ± 3.51	0.88 ± 2.97		
		R	10.926.67 ± 128.11	-2.19 ± 4.33	2.57 ± 2.39	3.35 ± 1.50	-0.34 ± 2.20	1.52 ± 4.20	1.25 ± 2.09	2.39 ± 2.99		

Abbreviations: DRN, dorsal raphe nucleus; HWL, hind paw withdrawal latency; L, left; OT, oxytocin; R, right. Control group microinjected with 5 μL of 0.9% saline. Comparisons among data at various time points in the same group were conducted with two-way repeated measures ANOVA. Comparisons of differences between groups were conducted using two-tailed *t* tests. Comparisons between left and right sides were conducted with paired *t* tests. Data expressed as mean ± S.E.M. **P < 0.01 and ***P < 0.001 compared with the control group at the corresponding time point.

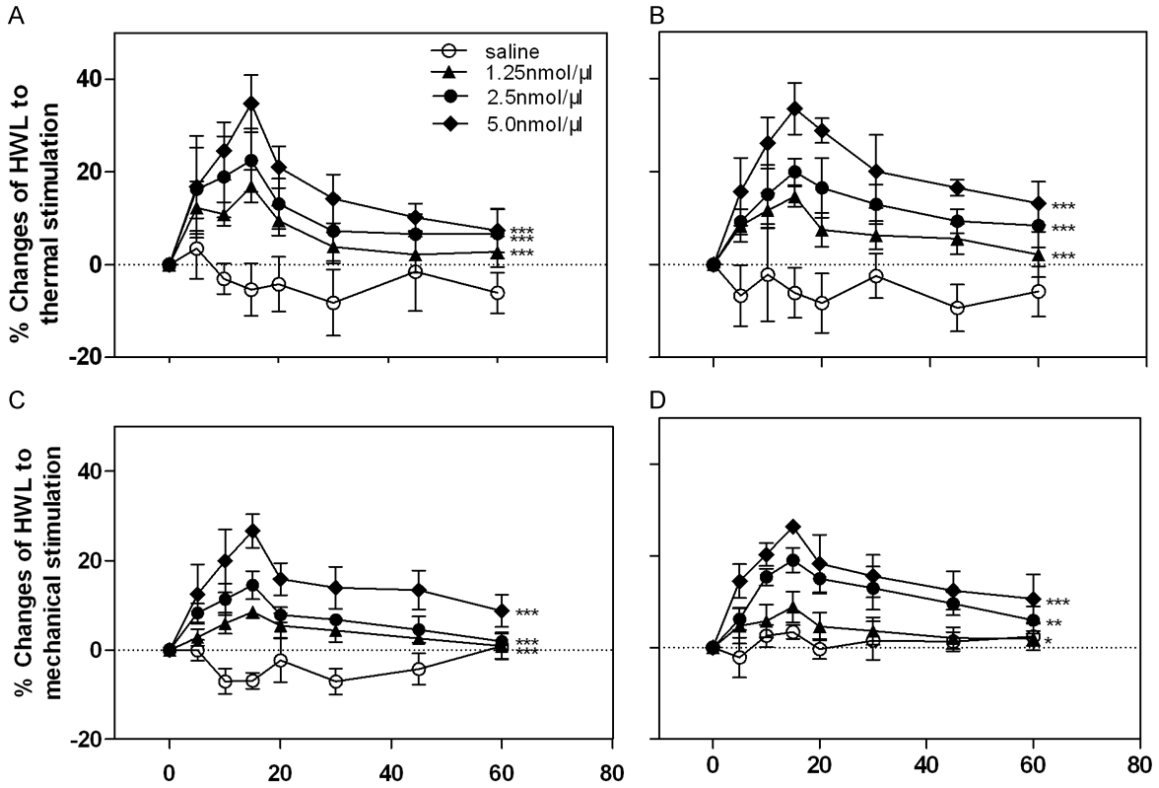

Figure 2. Percentage of HWL change in adult rats modeling neuralgia injected with saline or various concentrations of oxytocin (OT). Differences between groups were analyzed with one-way ANOVA. Pairwise data comparisons were conducted with least significant difference or Student-Newman-Keuls post hoc tests. *P < 0.05 vs. normal group. Effects of DRN OT microinjections at concentrations of 1.25, 2.5, or 5.0 nmol/μL on the HWLs to thermal (A and B) and mechanical (C and D) stimulation in rats modeling neuralgia. Control rats received a DRN microinjection of 1 μl of 0.9%. HWL indicates hind paw withdrawal latency. Data are presented as means ± S.E.M. The statistical significance of the difference between the groups was determined using one-way ANOVA.

nificant difference was observed in the HWLs to hot plate stimulation (P > 0.05), HWLs to pressure plate stimulation was significantly different between the two groups (P < 0.05). The results indicated that OT generated better analgesia during mechanical stimulation in adult rats modeling neuralgia than in normal adult rats (**Figure 3**).

Discussion

At present, analgesic drugs used for clinical applications include mainly opiates, such as morphine and dolantin. Although such drugs provide considerable analgesia in humans, they are also addictive; thus their long-term or high-dose use is limited [17, 18]. OT is a neuropeptide that exists in many parts of human body and has numerous functions [19, 20]. Interest in and research investigating the use of OT as a novel non-opioid analgesic have recently increased because OT appears to be without the adverse effects of opiates at the level of the central nervous system.

In the present study, the role of OT in pain regulation was investigated by measuring HWL as an indicator of pain in an adult rat model of neuralgia. The HWL to noxious thermal stimulation was determined using a hot plate, and the HWL to mechanical stimulation was measured using a pressure plate. The results indicated that HWLs to both noxious thermal stimulation and mechanical stimulation in the rats microinjected with various concentrations of OT into the DRN were significantly and dose-dependently increased compared with those in the control group (microinjected with saline). The effect was most apparent 15 min after the injection. This dose-dependent increase in HWL indicates that OT microinjected into the DRN generates significant analgesia in adult rats.

Table 6. HWL to Thermal or Mechanical Stimulation in a Rat Model of Neuralgia after microinjection of OT at a Concentration of 5.0 nmol/μL

Handling method		Side	HWL (s)	The percent change of HWL after trace injection in DRN of rats							F value	*P* value
				5 min	10 min	15 min	20 min	30 min	45 min	60 min		
Hot plate test	OT	L	320.00 ± 16.09	16.82 ± 10.98	24.53 ± 6.22	34.75 ± 6.14	21.02 ± 4.50	14.19 ± 5.28	10.22 ± 2.92	7.30 ± 4.58	22.62	0.000307***
		R	176.39 ± 72.01	15.66 ± 7.26	26.14 ± 5.50	33.52 ± 5.54	28.79 ± 2.62	20.02 ± 7.85	16.52 ± 1.71	13.14 ± 4.67	39.31	0.0000206***
	Saline	L	273.42 ± 35.84	3.48 ± 6.55	-3.07 ± 3.34	-5.39 ± 5.64	-4.22 ± 5.87	8.22 ± 7.13	-1.51 ± 8.55	6.09 ± 4.37		
		R	169.58 ± 9.85	-6.75 ± 6.59	-2.20 ± 10.02	-6.12 ± 5.38	-832. ± 6.37	-2.48 ± 4.77	-9.38 ± 5.05	-5.80 ± 5.40		
Pressure plate test	OT	L	12457.78 ± 5122.61	12.48 ± 6.64	19190 ± 7.09	26.62 ± 3.74	15.84 ± 3.57	13.91 ± 4.68	13.40 ± 4.35	8.75 ± 3.58	32.88	0.0000517***
		R	11258.89 ± 330.85	14.47 ± 3.68	20.27 ± 2.50	26.37 ± 1.13	18.29 ± 6.28	15.56 ± 4.64	12.42 ± 4.16	10.57 ± 5.38	23.66	0.000250***
	Saline	L	14180.83 ± 7.90.42	-0.09 ± 2.36	-7.07 ± 2.83	-6.89 ± 1.82	-2.34 ± 4.93	-7.07 ± 2.93	-4.29 ± 3.51	0.88 ± 2.97		
		R	10.926.67 ± 128.11	-2.19 ± 4.33	2.57 ± 2.39	3.35 ± 1.50	-0.34 ± 2.20	1.52 ± 4.20	1.25 ± 2.09	2.39 ± 2.99		

Abbreviations: DRN, dorsal raphe nucleus; HWL, hind paw withdrawal latency; L, left; OT, oxytocin; R, right. Control group microinjected with 5 μL of 0.9% saline. Comparisons among data at various time points in the same group were conducted with two-way repeated measures ANOVA. Comparisons of differences between groups were conducted using two-tailed *t* tests. Comparisons between left and right sides were conducted with paired *t* tests. Data expressed as mean ± S.E.M. ***P < 0.001 compared with the control group at the corresponding time point.

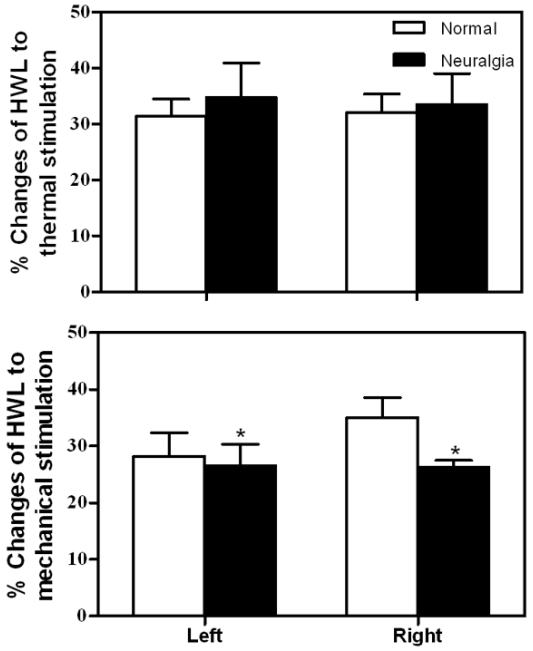

Figure 3. Percentage of HWL Change in Normal Adult Rats and Adult Rats Modeling Neuralgia 15 Min After Microinjection of Oxytocin (OT) at a concentration of 5 nmol/μL into the DRN. Differences between groups were analyzed with two-tailed *t* tests. Comparisons between the left and right sides were conducted with paired *t* tests. Comparison of the effects on the HWLs to thermal or mechanical stimulation between normal and neuralgia rats 15 min after DRN microinjection of OT at 5.0 nmol/μL. HWL indicates hind paw withdrawal latency. The statistical significance of the difference between the groups was determined using paired *t* tests.

In the adult rat model of neuralgia, DRN-microinjected OT at three different concentrations (1.25, 2.5 and 5 nmol/μL) led to different analgesic effects and to a significant increase in HWL. As shown in the tables and figures, increasing concentrations of OT enhanced the increased HWLs of the rats, indicating that OT microinjection into the DRN generated an analgesic effect in a dose-dependent manner. This result also provides a reliable pharmacologic basis for the clinical application of OT. Although its use as a non-opioid drug has attracted the interest of an increasing number of researchers, the mechanism for the analgesic effect of OT injected into the DRN in this rat model of neuralgia is unclear and requires further research.

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Disclosure of conflict of interest

None.

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References

[1] Zheng CX, Tu T, Guo BL, Wu YL, Luo SC, Sui BD, Dong SH and Wang YY. Dysfunction of mitochondria & chronic pain. Shenjing Jiepouxue Zazhi 2014; 30: 113-116.

[2] Xie YK. Pathogenesis of chronic pain. Chinese Sci Bull 1999; 44: 2353-2362.

[3] Pu YX and Shang HL. Clinical application of oxytocin. Zhonghua Zonghe Linchuang Yixue Zazhi 2007; 9: 91-92.

[4] Xu SF. Neurobiology. The 2nd edition. Shanghai: Shanghai Medical College Publishing Company; 1999. pp. 238-340.

[5] Carson DS, Guastella AJ, Taylor ER and McGregor IS. A brief history of oxytocin and its role in modulating psychostimulant effects. J Psychopharmacol 2013; 28: 15-17.

[6] Wan SX, Tang JQ, Hu QS, Yang Q and Wei JB. Influence of oxytocin on pain-evoked discharge of nucleus raphes magnus of adult rats. Hubei Zhongyi Xueyuan Xuebao 2001; 3: 23-24.

[7] Jurek B and Neumann ID. The oxytocin receptor: from intracellular signaling to behavior. Physiol Rev 2018; 98: 1805-1908.

[8] Bartz JA, Zaki J, Bolger N and Ochsner KN. Social effects of oxytocin in humans: context and person matter. Trends Cogn Sci 2011; 15: 301-309.

[9] Feifel D. Is oxytocin a promising treatment for schizophrenia? Expert Rev Neurother 2011; 11: 157-159.

[10] Agren G, UvnasMoberg K and Lundeberg T. Olfactory cues from an oxytocin-injected male rat can induce anti-nociception in its cagemates. Neuroreport 1997; 8: 3073-3076.

[11] Gupta S, Amrutkar DV, Mataji A, Salmasi H, Hay-Schmidt A, Sheykhzade M, Messlinger K, Olesen J and Jansen-Olesen I. Evidence for OT re-uptake in rat dura mater encephali. Br J Pharmacol 2010; 8: 1885-1898.

[12] Luo ZG, Han QG and Zhang XY. Research on chemical characteristics of immune cells in raphe nucleus of adult rats. Shenzhen Daxue Xuebao 2000; 17: 60-65.

[13] Mao JR and Yin QZ. Shape of dorsal raphe nucleus & its function in pain regulation. Suzhou Yixueyuan Xuebao 1989; 9: 70-73.
[14] Xie YF. Cytological structure & fiber connection of raphe nucleus. Heze Yixue Zhuanke Xuexiao Xuebao 2005; 12: 82-84.
[15] Sun YG and Yu LC. Progress in researches on participation of galanin in pain regulation. Zhongguo Tengtong Yixue Zazhi 2004; 10: 108-110.
[16] An K and Tian YK. Roles of galanin and its receptor in pain regulation. Guowai Yixue Mazuixue and Fusu Fence 2005; 26: 85-88.
[17] Zheng F, Yang L and Lei HY. Progress of researches on protection mechanism of preconditioning of opioids for liver ischemia-reperfusion injury. Linchuang Yiyao Shijian 2016; 25: 296-300.
[18] Huang XJ and Hou W. Abuse of addictive drugs, HIV infection & neural AIDS. Zhongguo Laonianxue Zazhi 2016; 36: 2814-2816.
[19] Feldman R. Oxytocin and social affiliation in humans. Horm Behav 2012; 61: 380-391.
[20] Strother LC, Srikiatkhachorn A and Supronsinchai W. Targeted orexin and hypothalamic neuropeptides for migraine. Neurotherapeutics 2018; 15: 377-390.