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Developmental change and sexual difference in synaptic modulation produced by oxytocin in rat substantia gelatinosa neurons



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ARTICLE INFO

Article history:

Received 4 April 2016

Received in revised form

9 June 2016

Accepted 13 June 2016

Available online 15 June 2016

Keywords:

Oxytocin

Excitatory transmission

Inhibitory transmission

Developmental change

Sexual difference

Spinal substantia gelatinosa

ABSTRACT

We have previously reported that oxytocin produces an inward current at a holding potential of -70 mV without a change in glutamatergic excitatory transmission in adult male rat spinal lamina II (substantia gelatinosa; SG) neurons that play a pivotal role in regulating nociceptive transmission. Oxytocin also enhanced GABAergic and glycinergic spontaneous inhibitory transmissions in a manner sensitive to a voltage-gated Na^+ -channel blocker tetrodotoxin. These actions were mediated by oxytocin-receptor activation. Such a result was different from that obtained by other investigators in young male rat superficial dorsal horn neurons in which an oxytocin-receptor agonist enhanced glutamatergic and GABAergic but not glycinergic spontaneous transmissions. In order to know a developmental change and also sexual difference in the actions of oxytocin, we examined its effect on spontaneous synaptic transmission in adult female and young male rat SG neurons by using the whole-cell patch-clamp technique in spinal cord slices. In adult female rats, oxytocin produced an inward current at -70 mV without a change in excitatory transmission. GABAergic and glycinergic transmissions were enhanced by oxytocin, the duration of which enhancement was much shorter than in adult male rats. In young (11–21 postnatal days) male rats, oxytocin produced not only an inward but also outward current at -70 mV, and presynaptically inhibited or facilitated excitatory transmission, depending on the neurons tested; both GABAergic and glycinergic transmissions were enhanced by oxytocin. The inhibitory transmission enhancements in adult female and young male rats were sensitive to tetrodotoxin. Although the data may not be enough to be estimated, it is suggested that synaptic modulation by oxytocin in SG neurons, i.e., cellular mechanism for its antinociceptive action, exhibits a developmental change and sexual difference.

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1. Introduction

A posterior pituitary hormone oxytocin produces a variety of actions such as social interaction and antinociception in addition to well-known milk ejection and uterine contraction (for review see [1,2]). Although there is much evidence for an involvement of oxytocin in antinociception at the spinal cord level (for review see [3,4]), this has not yet been examined thoroughly. We have previously reported in adult (6–8 weeks old) male rats that oxytocin produces a membrane depolarization without a change in glutamatergic excitatory transmission while enhancing GABAergic and glycinergic spontaneous inhibitory transmissions in spinal lamina II (substantia

gelatinosa; SG) neurons [5]. The SG neurons play a pivotal role in regulating nociceptive transmission from the periphery [6]. These oxytocin responses were mimicked by an oxytocin-receptor agonist [Thr⁴, Gly⁷]-oxytocin (TGOT) and inhibited by its antagonist [d(CH₂)₅¹, Tyr(Me)², Thr⁴, Orn⁸, des-Gly-NH₂⁹]-vasotocin. The depolarization was resistant to a voltage-gated Na^+ -channel blocker tetrodotoxin (TTX); the inhibitory transmission enhancements were depressed by TTX, indicating an involvement of an increase in neuronal activity [5]. On the other hand, in spinal superficial dorsal horn neurons of young (2–4 weeks old) male rats, TGOT has been reported to enhance glutamatergic and GABAergic spontaneous transmissions. Glycinergic spontaneous transmission was unaffected by TGOT [7]. Alternatively, the density of oxytocin-binding site in the superficial dorsal horn in newborn rats was higher than in adult rats [8,9]; there appeared to be a developmental change in the action of oxytocin on social interaction (for review see [10]). Thus, it is possible that oxytocin actions in the spinal dorsal horn exhibit a developmental alteration.

Abbreviations: PND, postnatal day; sEPSC, spontaneous excitatory postsynaptic current; SG, substantia gelatinosa; sIPSC, spontaneous inhibitory postsynaptic current; TGOT, [Thr⁴, Gly⁷]-oxytocin; TTX, tetrodotoxin; V_H , holding potential

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<http://dx.doi.org/10.1016/j.bbrep.2016.06.011>

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The role of oxytocin in social interaction exhibits sex differences. For instance, the intracerebroventricular administration of oxytocin promotes pair bonding in female but not male prairie voles ([11]; for review see [1,12]). Although there are sexual differences in the expressions of oxytocin and its receptor in the spinal dorsal horn [9], to our knowledge, it has not been examined whether oxytocin actions in the spinal dorsal horn exhibit sexual differences. In order to know a developmental change and sexual difference in the actions of oxytocin, we examined its effect on synaptic transmission in SG neurons of adult female and young male rat spinal cord slices by using the blind whole-cell patch-clamp technique and compared the results with those of adult male rats [5].

2. Materials and methods

All animal experiments were approved by the Animal Care and Use Committee of Saga University. Adult (6–8 weeks old) female and young [postnatal days (PNDs) 8–30] male rat spinal cord slice preparations were obtained in a manner similar to that described previously [5,13]. The slice was placed on a nylon mesh in the recording chamber, and was then completely submerged and superfused at a rate of 10–15 ml/min with Krebs solution which was saturated with 95% O₂/5% CO₂ and maintained at 36 ± 1 °C. The composition of Krebs solution used (in mM) was: 117 NaCl; 3.6 KCl; 2.5 CaCl₂; 1.2 MgCl₂; 1.2 NaH₂PO₄; 25 NaHCO₃; and 11 glucose (pH = 7.4). Whole-cell voltage-clamp recordings were made from SG neurons by using patch-pipettes fabricated from thin-walled, fiber-filled capillaries, as done previously [5,13]. The patch-pipette solutions used (in mM) to record spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs, respectively) contained: 135 K-gluconate; 5 KCl; 0.5 CaCl₂; 2 MgCl₂; 5 EGTA; 5 HEPES; 5 Mg-ATP; and 110 Cs₂SO₄; 0.5 CaCl₂; 2 MgCl₂; 5 EGTA; 5 HEPES; 5 Mg-ATP; 5 tetraethylammonium-Cl (pH = 7.2), respectively. The sEPSCs and sIPSCs were recorded at the holding potentials (V_{HS}) of –70 and 0 mV, respectively. GABAergic and glycinergic sIPSCs were recorded in the presence of strychnine (1 μM) and bicuculline (10 μM), respectively [5,14]. Signals were acquired using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Data were low-pass filtered at 5 kHz, and digitized at 500 kHz with an A/D converter (Digidata 1322A, Molecular Devices). The data were stored and analyzed with a personal computer using pCLAMP 8.1 software (Molecular Devices). sEPSCs and sIPSCs were detected and analyzed using Mini Analysis Program ver. 6.0.3 (Synaptosoft, Decatur, GA, USA); detection criteria for sEPSCs or sIPSCs included a 5 pA event threshold, their fast rise time (5, 10 and 4 ms for sEPSC, GABAergic and glycinergic sIPSCs, respectively) and a decay curve (20, 20 and 10 ms for sEPSC, GABAergic and glycinergic sIPSCs, respectively) that approximated to an exponential decay [5]. When sEPSC or sIPSC frequency and amplitude changed > 5% following superfusion of oxytocin, its effect was considered to be effective, as done previously [5]. When sIPSC frequency increase following oxytocin superfusion was measured in duration, the number of sIPSC events every 0.5 min in the absence and presence of oxytocin was plotted against time and then a time of period when the events increased > 5% compared to those before its application was calculated. Numerical data are given as the mean ± SEM. Statistical significance was determined as *P* < 0.05 using Student's *t* test. In all cases, *n* refers to the number of the neurons studied.

3. Results

3.1. Oxytocin action in adult female rat substantia gelatinosa neurons

At first, we examined the action of oxytocin (0.5 μM) on synaptic transmission in adult female rat SG neurons. Superfusing

oxytocin for 3 min produced an inward current at the V_H of –70 mV, as seen in Fig. 1(A). This inward current was seen in all neurons examined, as different from adult male rat neurons, 71% of which did so ([5]; Fig. 1(B)). The peak amplitude of this current averaged to be 9.9 ± 1.1 pA (*n* = 8), values comparable to those in adult male rats ([5]; Fig. 1(B)). This inward current declined in the presence of oxytocin (see Fig. 1(A)), as seen in adult male rat SG neurons [5]. On the other hand, oxytocin did not affect glutamatergic spontaneous excitatory transmission, as noted from Fig. 1(A). The frequency and amplitude of sEPSC, measured for 0.5 min, around 1.5 min after the beginning of oxytocin superfusion were, respectively, 104 ± 7% (*P* > 0.05) and 95 ± 3% (*P* > 0.05) of those (control: 12.1 ± 2.6 Hz and 11.5 ± 1.6 pA; *n* = 8) before its application (Fig. 1(C)).

Two kinds of GABAergic and glycinergic sIPSCs were recorded from adult female rat SG neurons, as reported previously in adult male rats (for example see [5,14]). As seen in adult male rats [5], each of the sIPSCs was enhanced in frequency and amplitude by oxytocin (0.5 μM) in all of the adult female rat SG neurons examined (see Fig. 2(A)). The enhancement in adult female rats was much shorter in duration than in adult male rats [5]. Duration times of the GABAergic and glycinergic transmission enhancements in adult female rats were 1.8 ± 0.4 min (*n* = 6) and 2.3 ± 0.7 min (*n* = 5), respectively. In 24 neurons where GABAergic transmission enhancement data obtained previously from adult male rats [5] were analyzed, 11 neurons exhibited a duration time of > 10 min and the other neurons had an averaged duration time of 4.0 ± 0.3 min (*n* = 13). These values were significantly larger than those of GABAergic transmission in adult female rats (*P* < 0.05). With respect to glycinergic transmission enhancements obtained from 27 neurons [5], 13 neurons exhibited a duration time of > 10 min and the other neurons had an averaged duration time of 4.4 ± 0.4 min (*n* = 14). These values were significantly larger than those of glycinergic transmission in adult female rats (*P* < 0.05).

At the peak of the enhancements, GABAergic sIPSC frequency and amplitude, relative to control (2.8 ± 0.7 Hz and 7.6 ± 0.3 pA; *n* = 6), were, respectively, 403 ± 52% (*P* < 0.05) and 172 ± 18% (*P* < 0.05); corresponding glycinergic ones were, respectively, 427 ± 110% (*P* < 0.05) and 223 ± 60% (*P* > 0.05; *n* = 5; control: 1.7 ± 0.4 Hz and 5.9 ± 1.3 pA). Each of the relative GABAergic and also glycinergic sIPSC frequency and amplitude was not significantly different in extent than that in adult male rats [GABAergic sIPSC frequency and amplitude: 538 ± 81% (*n* = 24) and 166 ± 9% (*n* = 20), respectively; glycinergic sIPSC frequency and amplitude: 678 ± 70% (*n* = 27) and 135 ± 7% (*n* = 19), respectively] which was measured around 2 min after the onset of oxytocin superfusion [5] (*P* > 0.05), except for glycinergic sIPSC amplitude. A significant difference in the amplitude may be due to the fact that the inhibitory transmission enhancement is due to not a direct action of oxytocin but its depolarizing effect leading to the production of action potentials (see [5]).

The facilitatory effects of oxytocin on GABAergic and glycinergic spontaneous transmissions in adult female rat SG neurons disappeared in the presence of TTX (0.5 μM; Fig. 2(B)). Under the pretreatment with TTX for 4 min, GABAergic sIPSC frequency and amplitude, measured for 0.5 min, around 1 min after the onset of oxytocin superfusion, relative to those just before its superfusion in the presence of TTX (2.4 ± 1.4 Hz and 7.2 ± 0.4 pA; *n* = 5), were, respectively, 110 ± 10% (*P* > 0.05) and 96 ± 6% (*P* > 0.05); corresponding glycinergic ones were, respectively, 114 ± 6% (*P* > 0.05) and 99 ± 7% (*P* > 0.05; *n* = 4; control: 1.0 ± 0.3 Hz and 10.1 ± 1.0 pA). These results indicate an involvement of an increase in neuronal activity in the facilitations, as seen in adult male rats [5].

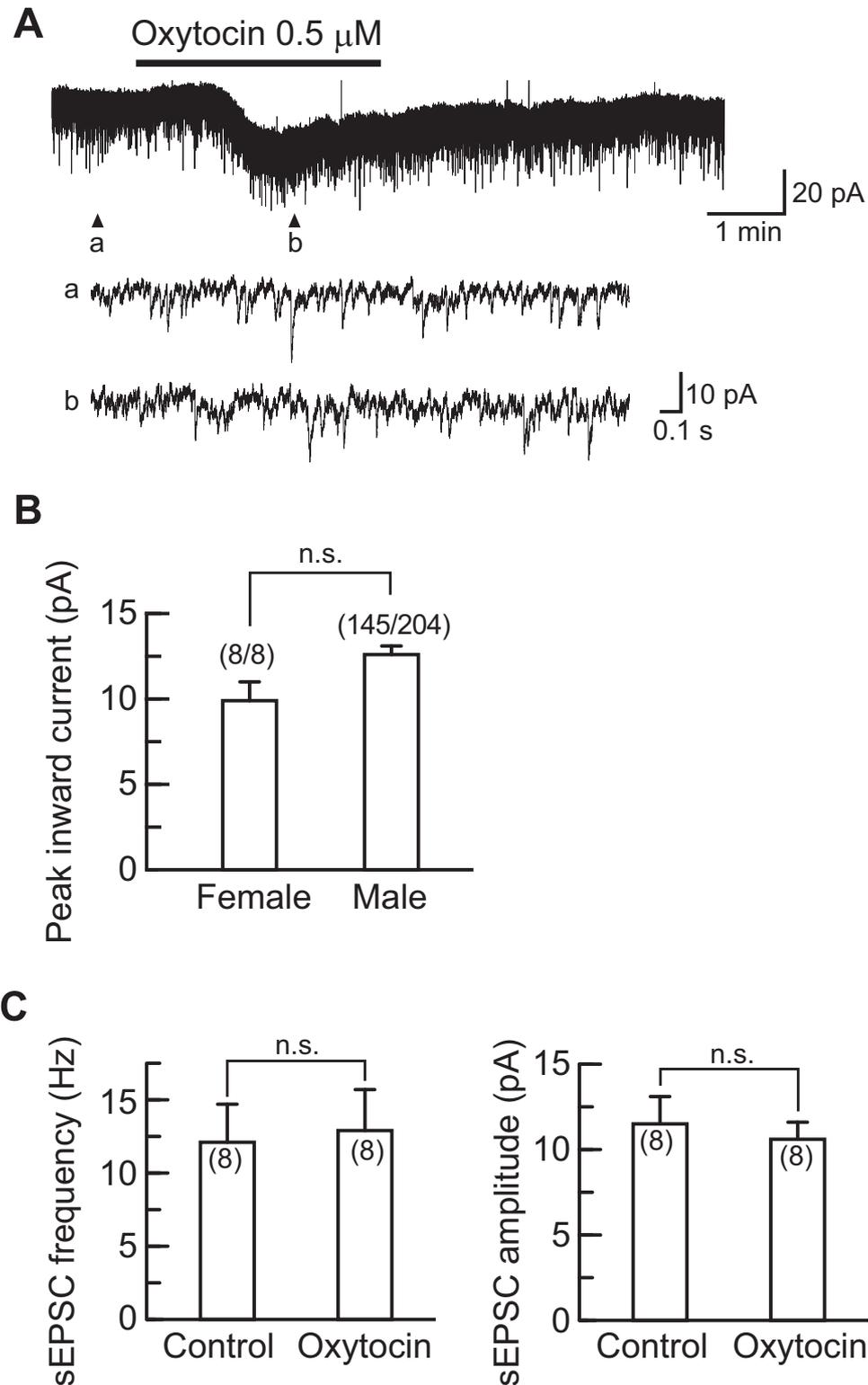


Fig. 1. Oxytocin (0.5 μ M) produces an inward current while unaffected glutamatergic spontaneous excitatory transmission in adult female rat substantia gelatinosa (SG) neurons. (A) Recordings of spontaneous excitatory postsynaptic currents (sEPSCs) in the absence and presence of oxytocin. In this and subsequent figures, the duration of drug superfusion is shown by a horizontal bar above the chart recording. (a) and (b) in this and subsequent figures showing sEPSCs: traces of spontaneous events for a period indicated by triangles, a and b, respectively, located below the chart recording, which are shown in an expanded scale in time. (B) Comparison of the peak amplitudes of the inward currents produced by oxytocin in adult female rat SG neurons with those of adult male rat SG neurons. The latter data were taken from [5]. Values in parentheses denote a ratio of the number of neurons in which oxytocin produces an inward current to that of all neurons examined. (C) Frequency and amplitude of sEPSC which were measured for 0.5 min in the control and around 1.5 min after the beginning of oxytocin superfusion. Values in parentheses indicate the number of the neurons tested; n.s.: not significant. Holding potential (V_H) = -70 mV.

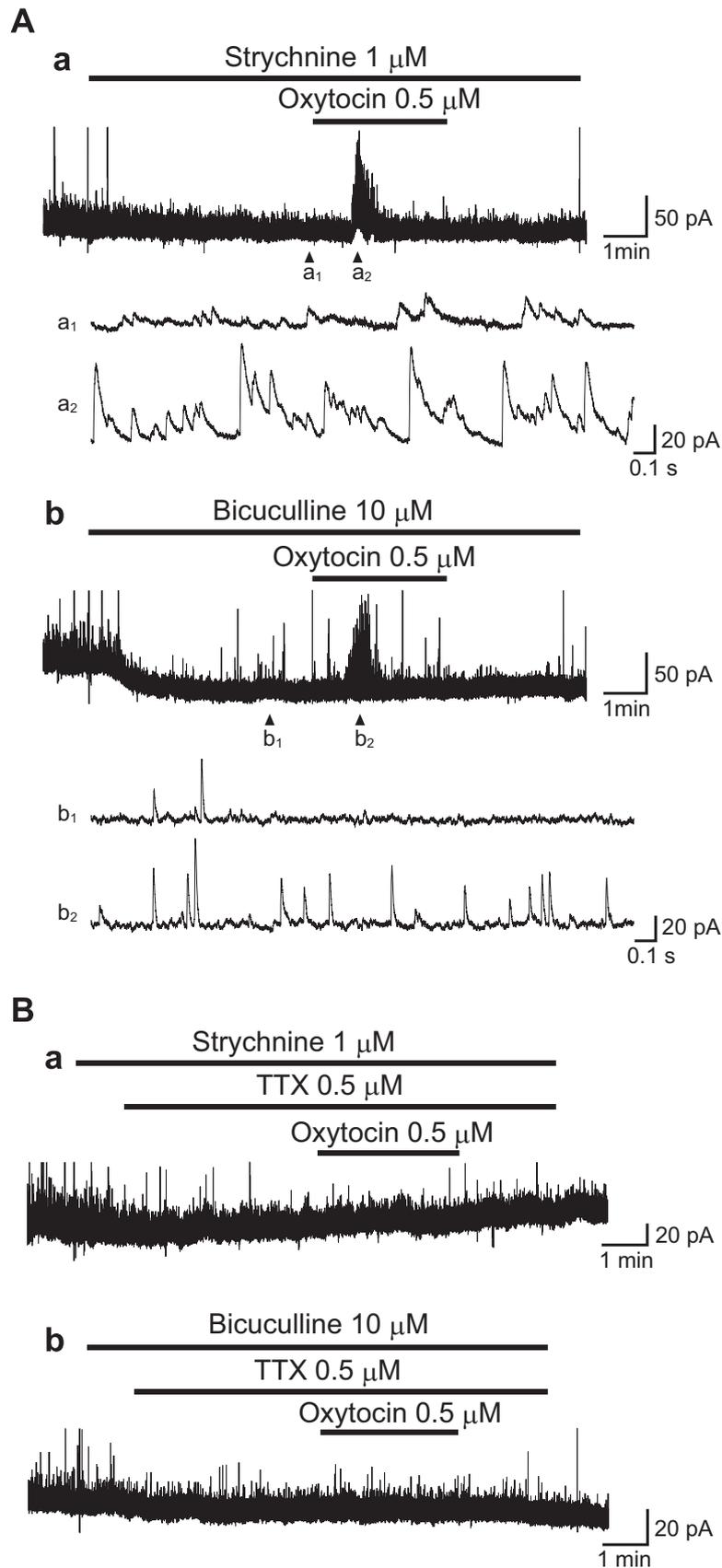


Fig. 2. Oxytocin (0.5 μM) increases the frequency and amplitude of spontaneous inhibitory postsynaptic current (sIPSC) recorded from adult female rat SG neurons in a manner sensitive to a voltage-gated Na^+ -channel blocker tetrodotoxin (TTX; 0.5 μM). (A, B) Recordings showing the effect of oxytocin on GABAergic (a) and glycinergic spontaneous inhibitory transmissions (b) in the absence (A) and presence of TTX (B). Each of the recordings in (A) and (B) was obtained from a different neuron. (a_1) and (a_2) in (Aa) [(b_1) and (b_2) in (Ab)] in this and subsequent Figs. showing sIPSCs: traces of spontaneous events for a period indicated by triangles, a_1 and a_2 (b_1 and b_2), respectively, located below the chart recording, which are shown in an expanded scale in time. Note in (Ab) and (Bb) that bicuculline itself produced a small inward current, as seen in adult male rat SG neurons [14]. $V_H=0$ mV.

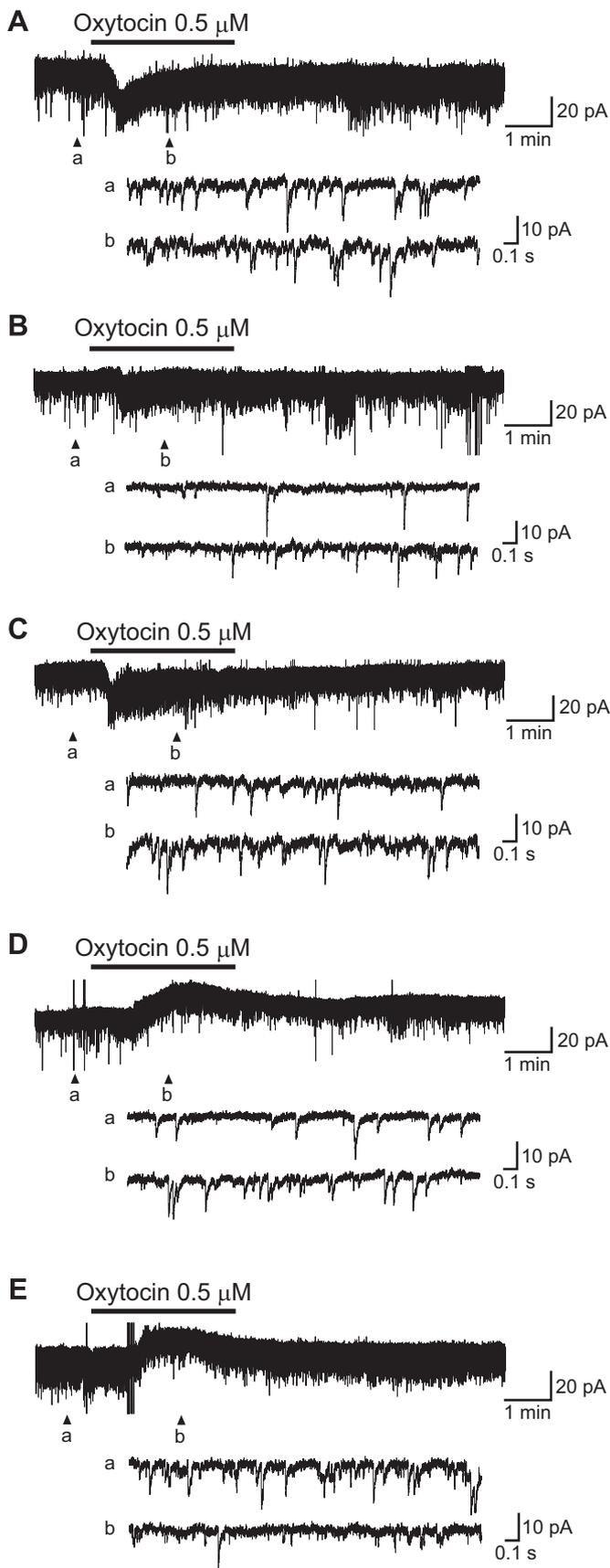


Fig. 3. Various patterns of changes in holding current and spontaneous excitatory transmission following oxytocin ($0.5 \mu\text{M}$) superfusion in young male rat SG neurons. (A–E) Chart recordings showing inward current only (A), sEPSC frequency increase only (B), both of them (C), both of outward current and sEPSC frequency increase (D) or decrease (E), produced by oxytocin. (A), (B), (C), (D) and (E) were obtained from postnatal day (PND) 13, 10, 14, 13 and 15 rat SG neurons, respectively. $V_H = -70 \text{ mV}$.

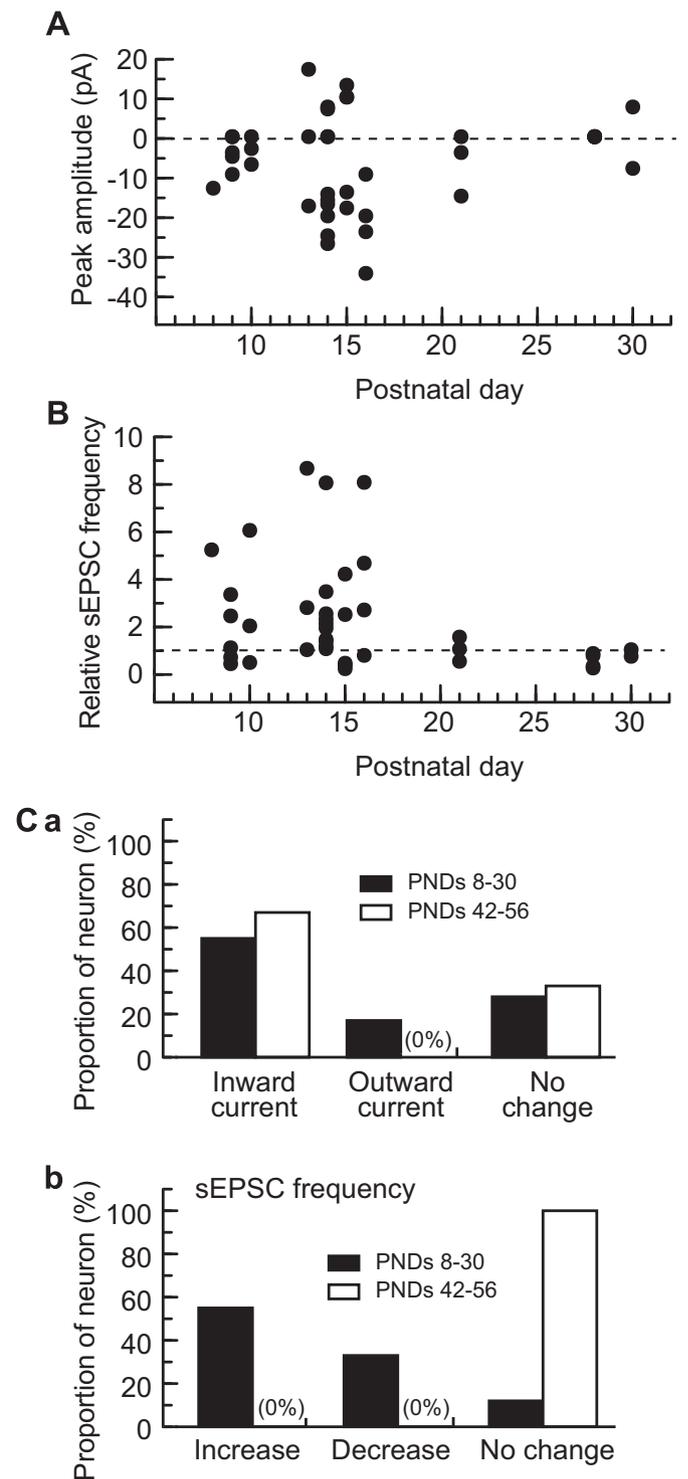


Fig. 4. Variation of changes in holding current and sEPSC frequency following oxytocin ($0.5 \mu\text{M}$) superfusion in young and adult male rat SG neurons. (A) Peak amplitude of outward or inward current produced by oxytocin which was plotted against PNDs in young rats. The outward and inward current amplitudes are given as positive and negative values, respectively. (B) sEPSC frequency, measured for 0.5 min, around 2 min after the beginning of oxytocin superfusion, relative to that before its superfusion, which was plotted against PNDs in young rats. (C) Comparison between young (8–30 PNDs) and adult (42–56 PNDs) rats in proportion of neurons exhibiting an inward or outward current (a) and an increase or decrease in sEPSC frequency (b). The data from adult rats were taken from [5].

3.2. Oxytocin action in young male rat substantia gelatinosa neurons

Next, we examined the action of oxytocin ($0.5 \mu\text{M}$) on synaptic transmission in young male rat SG neurons ($n=40$). Oxytocin

produced not only an inward current (Fig. 3(A), (C)), as seen in adult male rat SG neurons, but also an outward current (Fig. 3(D), (E)). In some of the SG neurons, there was almost no change in holding current (Fig. 3(B)). Fig. 4(A) demonstrates a change in holding current following oxytocin superfusion that is plotted against the age of rat used. Fifty-nine % and 27% of the 13–16 PNDs rat SG neurons tested ($n=22$) produced an inward and outward current, respectively. Their peak amplitudes averaged to be 19.8 ± 1.8 pA ($n=13$) and 10.8 ± 1.5 pA ($n=6$), respectively. The remaining neurons ($n=3$; 14%) exhibited almost no change in holding current.

With respect to excitatory transmission, the SG neurons tested exhibited an increase (Fig. 3(B), (D)) and decrease in sEPSC frequency following oxytocin superfusion (Fig. 3(E)), respectively, as different from adult male rat SG neurons. In only a small proportion of the neurons, there was no change in the transmission. Fig. 4(B) demonstrates a change in sEPSC frequency following oxytocin application that is plotted against the age of rat used. There was a tendency for sEPSC frequency to be increased in younger rather than older rats, albeit the data were not enough. Seventy-three % and 18% of the 13–16 PNDs rat SG neurons tested ($n=22$) produced an increase and decrease in sEPSC frequency, respectively. Their extents were on average $257 \pm 62\%$ ($P < 0.05$; $n=16$) and $60 \pm 12\%$ ($P < 0.05$; $n=4$), respectively, when measured for 0.5 min around 2 min after the beginning of oxytocin superfusion. These were accompanied by a small change in sEPSC amplitude, to be $133 \pm 7\%$ ($P < 0.05$; $n=16$) and $93 \pm 5\%$ ($P > 0.05$; $n=4$) of control, respectively. The remaining neurons ($n=2$; 9%) did not have any change in sEPSC frequency (97% and 101% of control). In these experiments, sEPSC frequency and amplitude before oxytocin superfusion were 6.9 ± 0.8 Hz and 8.3 ± 0.4 pA ($n=22$), respectively. Such an increase in sEPSC frequency was similar to that of TGOT's action observed by Breton et al. [7]. Although oxytocin responses show a developmental change, as noted from Fig. 4(A), (B), it may be useful to compare the responses in the total SG neurons tested between young and adult rats. Fig. 4 (C) demonstrates a comparison between young (8–30 PNDs) and adult (42–56 PNDs) male rats in the proportion of SG neurons exhibiting an inward or outward current and an increase or decrease in sEPSC frequency.

As reported by Baccei and Fitzgerald [15], both GABAergic and glycinergic sIPSCs were observed in all young (> 11 PND) male rat SG neurons examined. GABAergic sIPSCs in all of the 14–21 PNDs rat neurons tested were enhanced in frequency and amplitude by oxytocin (Fig. 5(Aa)), as shown for TGOT actions [7]. GABAergic sIPSC frequency and amplitude under the action of oxytocin, relative to control (3.8 ± 0.5 Hz and 10.0 ± 0.8 pA; $n=6$), were, respectively, $449 \pm 77\%$ ($P < 0.05$) and $136 \pm 14\%$ ($P < 0.05$), when measured for 0.5 min around 2 min after the onset of oxytocin superfusion. Although TGOT had no effect on glycinergic sIPSCs [7], oxytocin increased the frequency and amplitude of glycinergic sIPSC in all 11–19 PNDs rat SG neurons examined (Fig. 5(Ab)). Glycinergic sIPSC frequency and amplitude under the action of oxytocin, relative to control (2.5 ± 0.6 Hz and 12.3 ± 1.7 pA; $n=6$), were, respectively, $666 \pm 162\%$ ($P < 0.05$) and $139 \pm 15\%$ ($P < 0.05$), when measured for 0.5 min around 2 min after the onset of oxytocin superfusion. A reason for this discrepancy between our and Breton et al. [7]'s results is unknown. The GABAergic and glycinergic sIPSC frequency and amplitude increases in young male rats were not different in extent from those in adult male rats (see above; $P > 0.05$).

The facilitatory effects of oxytocin on GABAergic and glycinergic spontaneous transmissions in young male rat SG neurons were inhibited by TTX ($0.5 \mu\text{M}$; Fig. 5(B)). Under the pretreatment with TTX for 4 min, GABAergic sIPSC frequency and amplitude, measured for 0.5 min, around 2 min after the onset of oxytocin superfusion,

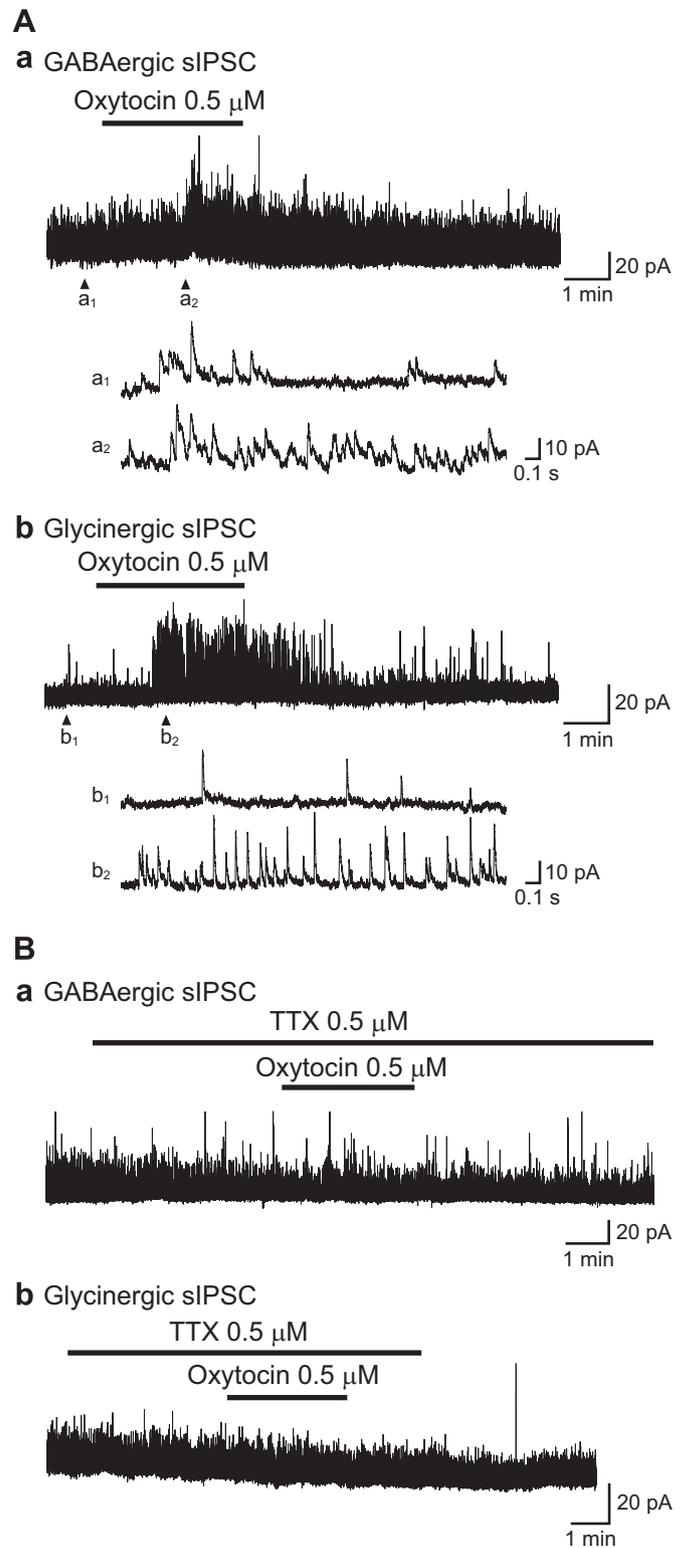


Fig. 5. Oxytocin ($0.5 \mu\text{M}$) increases the frequency and amplitude of sIPSC recorded from young male rat SG neurons in a manner sensitive to TTX ($0.5 \mu\text{M}$). (A, B) Chart recordings showing the effect of oxytocin on GABAergic and glycinergic spontaneous inhibitory transmissions in the absence (A) and presence of TTX (B). Each of the recordings in (A) and (B) was obtained from a different neuron; the recordings in (a) and (b) in both (A) and (B) were obtained in the presence of strychnine ($1 \mu\text{M}$) and bicuculline ($10 \mu\text{M}$), respectively. (Aa), (Ab), (Ba) and (Bb) were obtained from PND 21, 12, 14 and 11 rat SG neurons, respectively. $V_H=0$ mV.

relative to those just before its superfusion in the presence of TTX (1.5 ± 0.4 Hz and 9.9 ± 1.7 pA; $n=5$), were, respectively, $95 \pm 6\%$ ($P > 0.05$) and $95 \pm 1\%$ ($P < 0.05$); corresponding glycinergic ones

were, respectively, $114 \pm 14\%$ ($P > 0.05$) and $96 \pm 4\%$ ($P > 0.05$; $n = 5$; control: 1.5 ± 0.4 Hz and 9.4 ± 0.5 pA).

4. Discussion

We demonstrated in all of the adult female rat SG neurons tested that oxytocin produces an inward current at -70 mV without a change in glutamatergic spontaneous excitatory transmission and transiently enhances GABAergic and glycinergic spontaneous inhibitory transmissions. In young (13–16 PND) male rat SG neurons, we found out that oxytocin produces not only inward current (membrane depolarization; 59% of the neurons examined) but also outward current (hyperpolarization; 27%) at -70 mV. The remaining neurons (14%) exhibited almost no change in holding current (also see Fig. 4(Ca)). With respect to spontaneous excitatory transmission, oxytocin produced a pre-synaptic facilitation and inhibition which were seen in 73% and 18% of the neurons tested, respectively; the other neurons (9%) had no effect on excitatory transmission (also see Fig. 4(Cb)). On the other hand, both GABAergic and glycinergic transmissions in all young (11–21 PND) rat neurons examined were enhanced by oxytocin, although intracellular Cl^- concentration may change with development during the ages, as shown in rat spinal lamina I neurons [16]. The inhibitory transmission enhancements in adult female and young male rat SG neurons were sensitive to TTX, indicating an involvement of an increase in neuronal activity. This result was the same as that of adult male rat SG neurons [5].

4.1. Sexual difference in the action of oxytocin on synaptic transmission in rat substantia gelatinosa neurons

The inward current (depolarization) and no change in spontaneous excitatory transmission in adult female rats were similar to those observed in adult male rats [5]. There was not a difference in peak amplitude between the inward currents in female and male adult rats, although adult male rats had higher oxytocin-binding site densities in the superficial dorsal horn than adult female rats [9]. In female as well as male adult rat SG neurons, the inward current declined in amplitude in the presence of oxytocin, probably owing to a desensitization of oxytocin receptors or a degradation of oxytocin by peptidases [17]. Mechanisms for the current declination remain to be examined.

Although oxytocin increased GABAergic and glycinergic sEPSC frequency and amplitude in female rat SG neurons with extents comparable to those in male rats [5], the duration of the increase in female rats was much shorter than in male rats. This may be due to a distinction between male and female rats in a process of the spontaneous release of GABA and/or glycine from inhibitory SG neurons where oxytocin produces a membrane depolarization, because the extent of the depolarization does not differ between female and male rat SG neurons. The possibility cannot be ruled out that there is a difference in oxytocin receptor properties between male and female rats, because the synthesis of oxytocin and its receptor is reported to be partially estrogen-dependent [12] and oxytocin receptors are modulated by sex steroids [18]. Although the intrathecal administration of oxytocin produces antinociception in male rats [3]; for example see [19]), to our knowledge, there appear to be no reports about a difference between male and female rats in antinociception produced by oxytocin-receptor activation.

4.2. Developmental change in the action of oxytocin on synaptic transmission in male rat substantia gelatinosa neurons

Breton et al. [7] have reported in the young (2–4 weeks old) male rat superficial dorsal horn that 10% of the neurons tested

produce a membrane depolarization in response to TGOT ($1 \mu\text{M}$) and that the remaining neurons (90%) do not exhibit any change in membrane potential. The percentage of neurons exhibiting depolarization was less than that (59%) obtained from 13 to 16 PND rat SG neurons by using oxytocin ($0.5 \mu\text{M}$) in the present study. On the other hand, the present study showed a membrane hyperpolarization produced by oxytocin in 27% of the neurons tested (in 13–16 PND rats), an observation which was not reported by Breton et al. [7]. Such a hyperpolarization was seen in only 1% of the adult male rat neurons tested [5]. It is noted from Fig. 4(Ca) that oxytocin produces an outward current (hyperpolarization) only in young rat SG neurons. The variability in holding current change following oxytocin superfusion may be due to the fact that the SG is composed of a heterogeneous cell group of excitatory and inhibitory neurons [20] which may develop with time in a manner different from each other. For instance, many synaptic processes in young rats are still under maturation like those ensuring proper chloride homeostasis involved in the efficacy of inhibitory transmission (see [16]).

We have previously demonstrated that depolarization produced by oxytocin in adult male rats is due to a change in membrane permeabilities to K^+ and/or Na^+ , which is possibly mediated by phospholipase C and IP_3 -induced Ca^{2+} release [5]. It is well-known that oxytocin activates different intracellular pathways through the activation of the specific G proteins, resulting in opening/closing of ion channels [2]. It is possible that a developmental change in ion channels or cellular signaling cascades results in a variability of change in holding current produced by oxytocin with development. This issue remains to be examined.

Ninety-one % of the 13–16 PND rat SG neurons tested exhibited a change (increase or decrease) in sEPSC frequency following oxytocin superfusion, as different from that in adult rat SG neurons where there was no change in the frequency in all neurons examined ($n = 174$; [5]). This result can be clearly seen from Fig. 4(Cb). Although the number of neurons tested is not enough, it is noted from Fig. 4(B) that neurons exhibiting sEPSC frequency increase following oxytocin superfusion are decreased in number with development during 8–30 PND. Oxytocin may remarkably increase sEPSC frequency in only SG neurons of young (< 16 PND) rats.

sEPSC frequency increase and GABAergic transmission enhancement in young male rats, similar to those in the present study, have been reported by Breton et al. [7] for TGOT actions in superficial dorsal horn neurons. Based on the TGOT actions, they have proposed the idea that an increase in the spontaneous release of l -glutamate onto GABAergic neurons produced by oxytocin-receptor activation results in GABAergic transmission enhancement, a cellular mechanism for antinociception produced by oxytocin. Oxytocin actions similar to those of TGOT could contribute to a decrease in the excitability of SG neurons. Since inhibitory transmission enhancements produced by oxytocin in adult rat SG neurons were due to its depolarizing effect [5], there appeared to be a developmental change in mechanism for the production of the enhancement. Fig. 6 gives a schematic diagram showing possible mechanisms for the inhibitory transmission enhancements.

In addition to such an inhibitory transmission enhancement, the membrane hyperpolarization and sEPSC frequency (l -glutamate release) decrease, as revealed in the present study, would result in a decrease in the excitability of SG neurons, as seen in the actions of endogenous antinociceptive neuropeptides such as endomorphins [13] and nociceptin ([21,22]; for review see [23]). Thus, there may be a variety of mechanisms for antinociception produced by oxytocin in young male rats.

In the present study, the action of oxytocin on synaptic transmission in SG neurons was examined by superfusing this peptide in a spinal cord slice. Bath-applied oxytocin may have reached

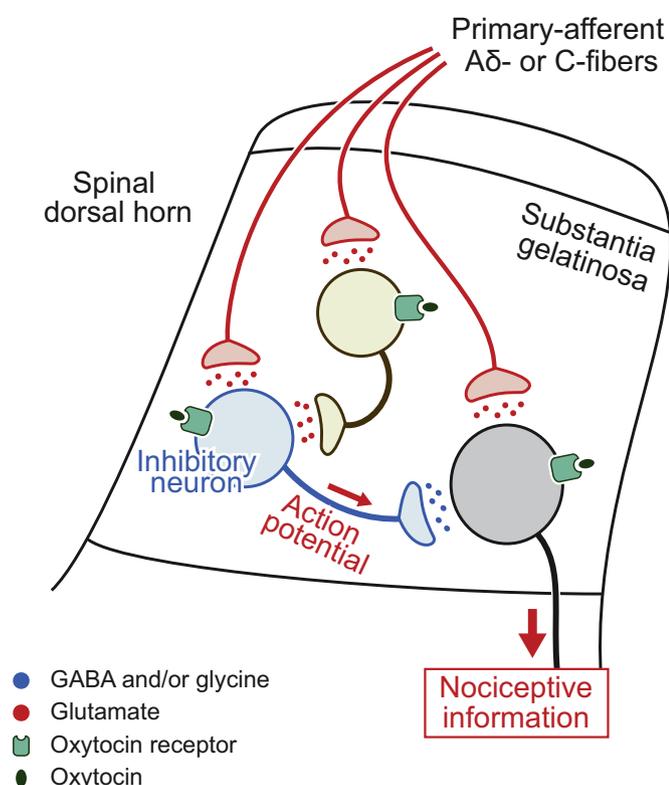


Fig. 6. Schematic diagram showing spontaneous inhibitory transmission enhancements produced by oxytocin in young and adult male rats. The activation of oxytocin receptors increases the activity of an inhibitory neuron that makes synapse with a neuron transferring nociceptive information in the substantia gelatinosa that plays a crucial role in regulating nociceptive transmission through primary-afferent A δ - and C-fibers from the periphery [6]. In adult rats, the occurrence of action potential in an inhibitory neuron is due to a membrane depolarization produced by the activation of oxytocin receptors located in the neuron [5]. In young rats, on the other hand, oxytocin-receptor activation increases the spontaneous release of L-glutamate onto an inhibitory neuron, resulting in the production of action potential in the neuron.

differentially excitatory or inhibitory SG neurons first to drive the final effect; this may be the reason why there are a variety of oxytocin responses. Alternatively, this variability may be correlated with global excitation/inhibition in the dorsal horn neuronal network after oxytocin superfusion. Martínez-Lorenzana et al. [24] have reported that the electrical stimulation of the anterior part of the hypothalamic paraventricular nucleus increases oxytocin concentration in cerebrospinal fluid and spinal cord tissues and produces antinociception in rats. In order to know what is a final response on SG neurons after physiological release of oxytocin, it would be necessary to be examined how synaptic responses recorded from SG neurons using the *in vivo* patch-clamp technique [25] are affected by electrically or optogenetically [26] stimulating hypothalamic neurons containing oxytocin.

In conclusion, although our data were preliminary, we demonstrated for the first time that mechanisms for antinociception produced by oxytocin change with development and that synaptic modulation by oxytocin in SG neurons exhibits a sexual difference in terms of the duration of spontaneous inhibitory transmission enhancement.

Acknowledgements

This work was supported by Grants-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (KAKENHI: 15K08673; 24500461).

Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bbrep.2016.06.011>.

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