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Electrophysiological properties of identified oxytocin and vasopressin neurones

William E. Armstrong[1], **Robert C. Foehring**[1], **Matthew K. Kirchner**[1], **Celia D. Sladek**[2]

[1]Department of Anatomy & Neurobiology and Neuroscience Institute, University of Tennessee Health Science Center, Memphis, Tennessee

[2]Department of Physiology and Biophysics, University of Colorado School of Medicine, Aurora, Colorado

Abstract

To understand the contribution of intrinsic membrane properties to the different in vivo firing patterns of oxytocin (OT) and vasopressin (VP) neurones, in vitro studies are needed, where stable intracellular recordings can be made. Combining immunochemistry for OT and VP and intracellular dye injections allows characterisation of identified OT and VP neurones, and several differences between the two cell types have emerged. These include a greater transient K^+ current that delays spiking to stimulus onset, and a higher Na^+ current density leading to greater spike amplitude and a more stable spike threshold, in VP neurones. VP neurones also show a greater incidence of both fast and slow Ca^{2+}-dependent depolarising afterpotentials, the latter of which summate to plateau potentials and contribute to phasic bursting. By contrast, OT neurones exhibit a sustained outwardly rectifying potential (SOR), as well as a consequent depolarising rebound potential, not found in VP neurones. The SOR makes OT neurones more susceptible to spontaneous inhibitory synaptic inputs and correlates with a longer period of spike frequency adaptation in these neurones. Although both types exhibit prominent Ca^{2+}-dependent afterhyperpolarising potentials (AHPs) that limit firing rate and contribute to bursting patterns, Ca^{2+}-dependent AHPs in OT neurones selectively show significant increases during pregnancy and lactation. In OT neurones, but not VP neurones, AHPs are highly dependent on the constitutive presence of the second messenger, phosphatidylinositol 4,5-bisphosphate, which permissively gates N-type channels that contribute the Ca^{2+} during spike trains that activates the AHP. By contrast to the intrinsic properties supporting phasic bursting in VP neurones, the synchronous bursting of OT neurones has only been demonstrated in vitro in cultured hypothalamic explants and is completely dependent on synaptic transmission. Additional differences in Ca^{2+} channel expression between the two neurosecretory terminal types suggests these channels are also critical players in the differential release of OT and VP during repetitive spiking, in addition to their importance to the potentials controlling firing patterns.

Keywords

afterhyperpolarisations; depolarising afterpotentials; ion channels; oxytocin; vasopressin

Correspondence: William E. Armstrong, Department of Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN. warmstro@uthsc.edu.

1 | INTRODUCTION

Action potentials generated in the cell bodies of magnocellular, hypothalamic vasopressin (VP) and oxytocin (OT) neurones result in depolarisation of their nerve terminals in the neurohypophysis, initiating exocytotic hormone release leading to important physiological changes in peripheral organs. Our report in the inaugural issue of *Journal of Neuroendocrinology*[1] was one of the first studies to temporally correlate electrical activity of supraoptic neurones with direct measurement of VP release from the neurohypophysis. Subsequent work from multiple laboratories over the past three decades has resulted in substantial insights into the ionic and cellular mechanisms underlying the activity both VP and OT neurones. This work is reviewed below.

In the 1970s, Wakerley and colleagues performed a series of incisive experiments on lactating female rats that demonstrated the relationship between the pattern of electrical discharge of putative OT and VP neurones and systemic neurohypophysial hormone release.[2] Key to these studies was the ability to antidromically identify supraoptic (SON) or paraventricular (PVN) neurones with neural stalk stimulation and monitor action potential discharge at the same time whilst correlating this discharge with the release of OT, monitored in real time by intra-mammary pressure. Approximately half of the neurones in either the SON or PVN emit a high-frequency burst (50–100 Hz) for 2–4 seconds every 5–10 minutes, preceding the precipitous increase in intra-mammary pressure as sociated with milk let-down by 15–20 seconds. This stereotyped, synchronised bursting pattern characterised putative OT neurones in both the SON or PVN, and was repeatedly observed by other groups successfully investigating its mechanisms.[3,4]

The conclusion that OT neurones were associated with milk ejection led to the obvious hypothesis that non-milk ejection neurones were VP neurones, and concurrent immunochemical studies established that both VP and OT neurones were found in both the PVN and SON.[5,6] When VP release was stimulated, these putative VP neurones adopted an asynchronous pattern of bursting, with bursts and interburst intervals in the order of 10–30 seconds.[2] For both OT and VP release, a phasic bursting pattern of electrical activity maximises hormone release.[7–10] Despite phasic bursting being the more efficient mode, both neurone types can also adopt a fast, continuous pattern of activity when stimulated (eg, with hyperosmolality) and both can exhibit a slow irregular pattern at rest. This latter pattern is common for OT neurones and, for VP neurones, the phasic vs continuous patterns may evolve depending on the intensity of stimulation.[2]

The electrical activity of OT and VP neurones in vivo depends on synaptic properties, as well as intrinsic membrane characteristics. In this review, we focus on the intrinsic properties of identified OT and VP neurones as assessed primarily from sharp electrode intracellular or whole-cell recordings from in vitro preparations. Although magnocellular OT and VP neurones also are found in the PVN, with smaller numbers in accessory neurosecretory nuclei, this review focuses on studies of the SON of rats, where the majority of this work has been conducted.

2 | MEANS OF IDENTIF YING OT AND VP NEURONES COUPLED WITH ELECTRICAL RECORDING

2.1 | Firing patterns

Identification of OT vs VP neurones based on the firing patterns found in vivo has obvious drawbacks, given that VP neurones exhibit both phasic and continuous patterns in vivo, and that the stereotyped, synchronous bursting of OT neurones is considered to be specific to lactating rats. A final caveat is that, even in vivo, a small percentage of milk-ejection neurones fired both synchronous milk ejection bursts, and exhibited asynchronous VP-like bursting inbetween bursts.[11,12]

Phasic bursting similar to that observed in putative VP neurones in vivo was observed in a variety of in vitro preparations in early research, such as acute[13–17] and organotypic[18] slices (Figure 1). Other types of activity, including slower, irregular firing, continuous firing, and even silent neurones, were often observed. A meta-analysis from Armstrong and Sladek[19] revealed a similar distribution of these different patterns in the acutely prepared hypothalamic explant but, quantitatively, the phasic bursting patterns observed corresponded well with those reported in vivo for putative VP neurones regarding burst length and intraburst frequency with minimal activation,[20] although they had had longer interburst intervals. Although there are many caveats to the assumption that phasic bursting in either the SON or PVN represents VP neurones exclusively, the probability is nevertheless high (see below), and the expression of bursting in vitro allowed investigations of its mechanisms, such as depolarising afterpotentials leading to plateau potentials,[21] as discussed below (Figure 1).

2.2 | Response characterisation

Additional tests can support cell identification in vivo. VP neurones can adopt, or enhance if already active, phasic bursting patterns in response to potent VP-releasing stimuli such as haemorrhage, water deprivation or carotid occlusion,[11,20,22–24] whereas putative OT neurones accelerate their continuous firing rate monotonically when stimulated (eg, with hyperosmotic challenge that also release OT).[2] A more reliable test for nonlactating female and male rats in vivo combines acute hypertension prompted by i.v. phenylephrine injection to selectively inhibit putative VP neurones, and systemic cholecystokinin (CCK) administration that activates gastric afferents and selectively excites OT neurones, similar to gastric expansion.[25,26] Unfortunately, these stimuli are not useful in in vitro preparations such as the hypothalamic explant, where CCK directly activates the majority of SON neurones and releases VP.[27]

Despite the many other neuroactive substances localised in magnocellular nuclei, only the responses to VP and OT appear to be useful in distinguishing the two cell types because each type selectively carries an appropriate autoreceptor.[28,29] VP[30,31] and OT[32,33] have been shown to specifically autoregulate their respective cell types via somatodendritically released peptide in vivo, where VP appears to be auto-inhibitory, and OT auto-excitatory. In vitro, direct application of OT or VP on dissociated SON neurones increases $[Ca^{2+}]_i$ selectively in the immunochemically identified parent cell type.[33–35] In slices, however,

although OT was found to excite nonphasic neurones preferentially over phasic (putative VP neurones) by Yamashita et al,[36] comparable differential effects of the two peptides on OT and VP neurones have not been reported.

2.3 | Immunochemical identification after intracellular dye injection

Although magnocellular neurosecretory subtypes were first labelled with specific antibodies following intracellular recording and Lucifer Yellow (LY) dye injection in goldfish by Reaves and Hayward,[37,38] no differences in the electrical properties or firing rates of the three different neurosecretory cell types found in fish were reported; thus, any comparison with the mammalian homologs in the SON and PVN is difficult. Yamashita et al[17] and Cobbett et al[39] immunochemically identified LY-injected SON neurones in rat hypothalamic slices, and found that the majority of phasic bursting neurones recorded could be labelled for VP or its associated neurophysin (VP-NP). Although these data were not compared with a similar labelling for OT neurones, nor were the nonphasic, continuously active neurones analysed in the same manner, the results nevertheless confirmed that phasically bursting neurones contained VP. For simplicity, VP or VP-NP immunoreactive neurones are referred to here as VP neurones. Similar experiments studying OT neurones have used antibodies either to OT or to its specific neurophysin (OT-NP).

Erickson et al[40–42] first successfully immunolabelled both cell types following recording and biocytin injection in slices from guinea pig SON. Phasic bursting was only found in VP neurones, and only those exhibiting depolarising afterpotential (DAPs).[41,42] Although OT neurones did not show this behaviour, the phasic bursting in guinea pigs demonstrated much shorter bursts, and shorter interburst intervals than those reported in rats. Armstrong et al[43] found that phasic bursting patterns in the SON from rat hypothalamic slices was similar to that recorded in vivo[43] (Figure 1), and was more often associated with biocytin filled, VP neurones; however, phasic bursting was observed in a few OT neurones, just as in vivo. In organotypic cultures, biocytin-injected VP neurones were found to fire in phasic bursting patterns asynchronously and, remarkably, OT neurones exhibited synchronous discharges mimicking those associated with milk ejections in vivo.[45]

In dissociated SON neurones, differential OT and VP immunolabelling is also possible after recording, if more difficult, allowing characterisation of many electrophysiological properties after recording.[33,46–49]

In single neurohypophysial terminals, which contain large quantities of hormone, investigators have immunoblotted terminal contents for VP and OT after recording,[50] or used an enzyme-linked immunoassay.[51] These methods have been valuable in determining many properties of OT and VP terminals following recording.[52]

2.4 | Reverse transcriptase-polymerase chain reaction (RT-PCR)

Single cell RT-PCR has been used to categorise membrane channels and other gene products of OT and VP neurones,[53–57] and has been applied following patch clamp characterisation of SON neurones.[58] However, the sensitivity of this technique coupled with the known co-localisation of both OT and VP mRNAs in many SON neurones[54] demands either real-time single-cell RT-PCR[58] or some other means of quantification to distinguish VP-dominant

from and OT-dominant neurones).[54] The much smaller numbers of co-localised VP-OT neurones observed with immunochemistry suggests a lesser degree of protein co-expression. This observation matches physiological studies showing selective release of OT (eg, during the milk ejection response),[2] despite over 50% of SON neurones containing both mRNAs.[54]

2.5 | Transgenic rodent lines with fluorescent markers

Post-recording identification can be avoided altogether by using transgenic rats in which the VP[59] or OT[60,61] promoters control the expression of different fluorescent transgenes. For example, these transgenic strains have been used to identify differences in the two cell types with respect to acid-induced currents[62] and Ca^{2+} oscillations.[63] Although co-localisation is still an issue, cross-breeding these strains allows the focus to be on neurones exhibiting only one of the fluorescent markers, in much the same way that immunochemical studies are more definitive when positive neurones are negative for the heterotypic peptide.

3 | PROPERTIES OF IDENTIFIED OT AND VP NEURONES

3.1 | Passive electrical properties

Neuronal input resistance and membrane time constant are passive properties that contribute to the efficacy of synaptic inputs and their eventual summation at the spike initiating zone. No significant differences were reported in either input resistance (200–300 MΩ) or membrane time constant (11–16 milliseconds) for sharp electrode recordings from the two cell types in either male[43] or virgin and lactating female rats.[64,65] As expected, with whole-cell patch recording, input resistance was three to four times higher, and membrane time constant approximately twice as long in virgin and lactating rats but, again, no differences were observed across state or cell type[66] (but see also Li et al[44]). Thus, it is unlikely that the differences in the passive properties of OT and VP neurones contribute greatly to any differences in electrical activity.

3.2 | Action potentials (APs) or spikes

The frequency and patterning of APs determine hormone release. Thus, AP properties may contribute to the different patterns of OT and VP neuronal firing. In male rats, no differences were found in AP amplitude (approximately 75 mV) or half width (approximately 1.5 milliseconds), although spike broadening during AP trains was greater in VP neurones.[43] In female rats, spike threshold was more depolarised and spike heights were smaller in OT neurones.[64,65] Action potential widths increase during lactation, primarily, but not exclusively, in OT neurones.[55,64] The wider spikes reflected slower rise and decay times.[64] Similarly, frequency-dependent spike broadening characterises both cell types in female rats, although it is greater in OT neurones during pregnancy and lactation. The underlying mechanism for this difference is unknown but may relate to the weaker expression of Na^+ currents and repolarising, A-type K^+ currents in OT neurones (see below).

In adult female virgin rats, VP neurones have twice the Na^+ current density compared to OT neurones, although no differences were found in voltage dependence or the kinetics of various aspects of activation and inactivation with whole-cell recordings in dissociated neurones[49] (Figure 2). VP neurone spikes were larger in slice recordings, with a faster-rising

slope compared to OT neurones.[49,64] The difference in Na^+ channel density in the two cell types may underlie the observation that spike threshold in OT neurones was increased with steady-state depolarisation (−65 to −55 mV) but was unaffected in VP neurones.[49] In situ hybridisation and immunochemical studies show that SON neurones have tetrodotoxin (TTX)-sensitive $Na_V1.2$, $Na_V1.6$, and $Na_V1.7$ channels, as well as associated β1 and β2 accessory subunits and, although no differences were reported between cell types in male rats, mRNA and protein levels for these subunits were up-regulated by hypertonic saline.[67,68] A TTX-insensitive Na^+ channel, $Na_V1.9$[69] is also found in SON neurones but was not increased by salt loading.[69] The K^+ and Ca^{2+} currents that contribute to action potential shape are dealt with below.

3.3 | Spike afterhyperpolarisations (AHPs)

3.3.1 | Fast AHPs—Single APs in SON neurones are followed by an AHP lasting 10–20 milliseconds that gates firing rate by setting a minimum interspike interval. Multiple channels likely contribute to the fast AHP (fAHP), including a classical delayed rectifier K^+ current and an A- type current, which, along with Na^+ current inactivation, contribute to spike repolarisation. Large conducting, voltage- and Ca^{2+}-dependent BK channels[70,71] also contribute to the fAHP amplitude and duration but not to spike repolarisation *per se*.[72,73] Although some differences between OT and VP neurones exist in the currents underlying fAHPs, the potentials themselves are similar in amplitude and duration in both cell types.[43,64]

3.3.2 | Medium AHPs—The medium AHP (mAHP) in SON neurones lasts 200–500 milliseconds, and is carried by a Ca^{2+} -dependent K^+ current.[74] This mAHP accumulates with successive spikes in a train and is highly sensitive to the bee venom, apamin.[41,75–77] Apamin binds with high affinity to small conductance (SK), Ca^{2+}-dependent K^+ channels that are found in the SON.[78–82] Based on apamin sensitivity[43,83,84] and immunocytochemistry,[82] both cell types have SK channels (SK3 subtype), and apamin sensitivity accounts for approximately 80% of mAHP amplitude.[43,84,85]

The two cell types do not differ in the amplitude or decay of the mAHP in male[43] or virgin female rats.[64] By contrast, the mAHP in OT neurones increases and is associated with stronger spike frequency adaptation during pregnancy[65] and lactation,[64,65] suggesting that the enhanced bursting and excitation of OT neurones needed during reproduction is coupled with intrinsic restraints that limit the excitation to short periods of intense activity. The density of the underlying, apamin-sensitive current (I_{mAHP}) is likewise increased in lactation, independent of any changes in Ca^{2+} current density or $[Ca^{2+}]_i$.[83] This increase in I_{mAHP} current density may rest in part as a result of changes in the calmodulin-SK channel complex, mediated by changes in the α subunit of the calmodulin-SK binding protein, casein kinase 2 (CK2). CK2 is down-regulated during pregnancy[86] and, because CK2 typically phosphorylates calmodulin to reduce its Ca^{2+} sensitivity,[87] this decrease would in turn allow increased activation of the coupled SK channels.

There is also a striking difference in the biochemical regulation of the mAHP between the two cell types. The mAHP in OT neurones, but not VP neurones, is dependent on the

constitutive presence of the lipid, phosphatidylinositol 4,5-bisphosphate (PIP_2),[84] suggesting that the two cell types could differ in neuromodulation when the PIP_2 pathway is targeted (Figure 3). The difference appears to relate not to the interaction of PIP_2 with the SK3 channel but, more likely, to the modulation of high voltage-gated N-type Ca^{2+} channels, which supply the Ca^{2+} activating the mAHP.[84,88] It is not known whether this difference plays a role in the mAHP plasticity of OT neurones.

3.3.3 | Slow AHPs (sAHPs)—A slower Ca^{2+}-dependent AHP, the sAHP (1–2 seconds) also characterises SON neurones.[83,89,90] The sAHP requires longer (or higher frequency) spike trains to reach its maximum compared to the fAHP or mAHP.[90] As with the mAHP, the sAHP increases during pregnancy and/or lactation only in OT neurones.[83,91] The extent of the sAHP is best appreciated when blocking the temporally overlapping slow depolarising afterpotentials (sDAP) with Cs^+.[90] The strong inhibition of the sAHP with muscarine,[90] which operates on muscarinic receptors Gαq-coupled to the PIP_2 pathway in the SON,[92] suggests that cholinergic transmission could differentially target OT and VP neurones. As with the mAHP, Kirchner et al[84] also found a strong dependence of the sAHP on constitutively expressed PIP_2 in OT, but not VP neurones.

The channel underlying the sAHP is unknown. Greffrath et al[89] were able to suppress the sAHP with charybdotoxin, which blocks some BK, intermediate conductance, and Ca^{2+}-dependent K^+ channels; however, the sAHP was not isolated from the time-overlapping sDAP. However, in another study when the sDAP was first blocked, both charybdotoxin and iberiotoxin failed to inhibit the sAHP,[90] raising the possibility that Greffrath et al[89] may have been modulating the sDAP. Like the mAHP, the sAHP in OT neurones depends on PIP_2 expression and appears largely to be activated by Ca^{2+} from N-channels.[84,88] In VP neurones, sAHPs are suppressed by R-type Ca^{2+} channel blockers.[88]

3.4 | Depolarising afterpotentials (DAPs)

3.4.1 | Slow depolarising afterpotentials (sDAPs)—sDAPs following spikes were the first specific membrane property ascribed to either cell type, and their occurrence increases the probability of APs occurring in close succession (Figure 1). Andrew and Dudek[21,93] first reported sDAPs in the SON in association with the phasic bursting pattern typical of VP neurones in hypothalamic slices. The sDAP lasted 1–3 seconds (≤5 mV), could be observed after a single spike and summated to a plateau potential with repetitive spiking that supported long bursts of action potentials. sDAPs are activity- and Ca^{2+}-dependent, independent of TTX-sensitive Na^+ channels or synaptic transmission[21,43,77,94] and, although more often associated with VP neurones, are present in a minority of OT neurones.[43,45,64,65,95] Importantly, the sDAP can be masked by AHPs, and vice versa, making the study of specific spike afterpotentials difficult without pharmacological treatment.[43,90,96] However, although a few OT neurones with sDAPs can adopt phasic bursting reminiscent of VP neurones, most do not.[43,45] sDAPs do not underlie the synchronous, milk-ejection type bursting activity of OT neurones observed in organotypic cultures.[45] Thus, VP neurones appear to have a different mechanism for the sDAP and the plateau potential compared to OT neurones.

sDAP currents show strong voltage dependence starting at approximately −80 mV and increasing near spike threshold. Different mechanisms have been proffered. The seminal study by Bourque[94] suggested an inward cation current with a region of negative resistivity at approximately −65 mV consistent with a regenerative current. By contrast, Li and Hatton[97] reported a decreased K^+ conductance, consistent with the increased input resistance observed by many in current clamp.[77,94,98] Modelling studies show that either mechanism faithfully reproduces a sDAP and its summation to a plateau potential, provided that there is a resting Na^+ leak current,[72,99] consistent with the Na^+ dependence of the sDAP,[97] and also with its sensitivity to nonselective cation channel blockers.[100,101] sDAPs are inhibited by L- and N-type Ca^{2+} channel blockers and by reducing internal Ca^{2+} stores.[102]

sDAPs and the resulting plateau potentials underlying bursts are inhibited by κ-opiate receptor activation, an autocrine event driven by the activity-dependent, somatodendritic release of dynorphin from VP neurones.[103–105] This inhibition may work by decoupling the sDAP/plateau mechanism from its Ca^{2+} dependence.[99] Histamine, which promotes phasic bursting,[106] also enhances the sDAP in VP neurones via H_1-receptor activation.[77]

3.4.2 | Fast DAPs (fDAPs)—fDAPs (approximately 200 milliseconds) are also activity-, voltage- and Ca^{2+}-dependent, and are found in most (80%) VP neurones and less frequently (20%) in OT neurones.[96] The underlying current is consistent with a nonselective cation current and may be critical in initiating bursts. Unlike the sDAP, however, the fDAP is insensitive to Cs^+ blockade. An analysis of spike patterning of VP neurones in vivo suggests a prominent spike excitatory afterpotential with a time course similar to the fDAP, whereas the sDAP appears more dominant in vitro.[107] VP neurones, but not OT neurones, are immunoreactive for the transient receptor potential melastatin channel 5 (TrpM5), whereas both cell types are immunoreactive for TrpM4.[108] Elsewhere, these channels underlie transient, Ca^{2+}-dependent cation ion currents which have characteristics similar to those of the I_{fDAP}[96]

3.5 | Voltage-dependent Ca^{2+} channels

3.5.1 | High-threshold Ca^{2+} currents—Pharmacological investigations indicate the presence of high voltage-activated (HVA) L-, N-, and P/Q channels in most SON neurones in dissociated cell preparations[109–111] and, more recently, in identified OT and VP neurones.[88] Molecular studies have revealed expression of all HVA channel subunits in both cell types except α1E ($Ca_v2.3$, or R-type) channels.[53] Although considered as HVA-type currents, R-type currents may have a lower voltage dependence and faster inactivation kinetics than L, N or P/Q channels.[112]

N-type followed by L-type channels account for most of the whole cell Ca^{2+} current,[109,110] as well as the increases in $[Ca^{2+}]_i$ measured with imaging following spike trains[88]; neither channel type appears to differ between cell types. Similarly, although P/Q channels contribute a relatively smaller percentage of either Ca^{2+} current[109,110] or spike induced increases in $[Ca^{2+}]_I$, they do not appear different between OT and VP neurones. However, the R-type channel contribution may be more prominent in VP neurones,[113] which may account for its stronger relationship with the sAHP in those neurones.[88] This is in contrast to

neurohypophysial terminals, where R-channel blockers inhibited a portion of OT, but not VP, release that was resistant to L-, N- and P/Q blockers.[114]

A splice variant for Cav2.1 (P/Q type) lacking the so-called synprint motif that interacts with exocytotic proteins is present in the SON.[115] This variant appears to be exclusively related to OT neurones and terminals[116] and may relate to the inability of P/Q channel blockers to influence OT release to the same degree as they do VP release, either from terminals[117] or from the somatodendritic region.[113] Tobin et al[113] found N-channels to be more reliably coupled to local OT release than any other channel type. Both L- and N-type channels are important for terminal hormone release.[114,117]

3.5.2 | Low-threshold Ca^{2+} currents—The presence of low-threshold, transient, T-type Ca^{2+} currents in magnocellular neurones has been inconsistently reported, although studies have suffered from a lack of specific blockers and the possibility of confusion with R-channels that activate at more negative potentials than L, N, or P/Q HVA channels. Based on voltage threshold, a transient current and sensitivity to Ni^{2+}, T-like channels have been reported in SON neurones in some studies[41,48,109,118,119] but not others.[110,111,120] This inconsistency makes it likely that a type of low-threshold Ca^{2+} channel is at least minimally present, although detection may be dependent on particular recording conditions. Erickson et al[41] reported no difference between OT and VP SON neurones in guinea pig slices in the presence of a low-threshold, transient Ca^{2+} current. To date, there is no molecular evidence for the presence of CaV3 (Cacna1g-i: T-type) or CaV2.3 (Cacna1e: R-type) channels specifically in magnocellular OT or VP neurones. In subclasses of parvocellular PVN neurones, however, well described low-threshold Ca^{2+} currents[120–123] are likely to be a result of T-type (Cacna1 g, h) and possibly R-type (Cacna1e) Ca^{2+} channels.[124]

3.5.3 | Plasticity of Ca^{2+} channels—Although whole-cell Ca^{2+} currents in OT neurones are larger during lactation because of cell size increases, current density does not change.[83] It is nevertheless possible that specific Ca^{2+} currents could increase relative to a decrease in others. In dissociated SON neurones, spontaneous Ca^{2+} oscillations, although found in both cell types, are more prevalent in VP than OT neurones of virgin rats, whereas approximately twice as many OT neurones dissociated from lactating rats show these oscillation[63] and this could be channel specific.

After 24 hours of dehydration, L-channel Ca^{2+} density accounts for an increase in Ca^{2+} current density in both OT and VP neurones.[125] Water deprivation or salt loading not only activates both OT and VP neurones, but also leads to many morphological, electrophysiological and synaptic changes in magnocellular neurones and associated glia similar to those observed specifically in OT neurones during lactation.[126–128]

3.6 | Voltage-dependent K^+ channels

3.6.1 | A-type currents—SON and magnocellular PVN neurones have a prominent A-type current (I_A), comprising a K^+ current that activates rapidly at low voltage and decays rapidly.[47,73,120,129–134] The I_A underlies the prominent transient outward rectification (TOR) that delays spike activation in magnocellular PVN and SON neurones when depolarised from hyperpolarised membrane potentials, contributes to spike repolarisation, and likely

plays a significant role in somatic and terminal spike broadening (ie, which facilitates Ca^{2+} influx) via its activity-dependent inactivation[47,129,130,135,136]. The strong presence of TOR in the magnocellular neurones distinguishes them from many surrounding parvocellular neurones in the PVN[122] and SON.[137,138] Most SON neurones are immunopositive for an A-type channel subunit, KV4.2.[139] In the PVN, single cell RT-PCR studies show that approximately 75% of magnocellular VP and OT neurones I_A have mRNA for KV4.3 channels, although approximately 33% also have Kv4.2.[140] Unfortunately, because of the extensive co-localisation of OT and VP mRNA, discerning a difference between two types was not possible in these studies.

In current clamp studies, the TOR is stronger in VP than in OT neurones and this difference results in a significant delay to spiking from hyperpolarised membrane potentials[47,64] in VP neurones. This difference can be directly related to the greater density of I_A under voltage clamp in VP neurones, reflected by both peak amplitude, and in a larger contribution from a slowly inactivating component of the current[47] (Figure 4). Together, these studies suggest that OT neurones would be more excitable than VP to depolarising inputs in the range where I_A is active.

In another study of dissociated neurones, however, identified VP neurones were found to exhibit no I_A whatsoever compared to its presence in every identified OT neurone.[46] Fisher et al[47] suggested that, because Widmer et al[46] blocked Ca influx with Co^{2+} to unmask I_A from voltage-dependent Ca^{2+} currents, this result could be attributed to a differential Ca^{2+}-dependence between the two neurone types, a dependence shown in unidentified SON neurones.[129] However, the apparent Ca^{2+}- dependence has been at least partly attributed to charge screening effects of different divalent cations on voltage dependence because I_A is prominently displayed in Ca^{2+}-free media[73,132,133] and its voltage dependence is shifted to more depolarised potentials as Ca^{2+} is increased.[133] This is also consistent with the strong suppression of I_A in PVN magnocellular neurones by Co^{2+}, but not in Ca^{2+}-free media.[132] Regardless, the dramatic absence of I_A in VP neurones reported by Widmer et al[46] suggests that there may be some important difference in its regulation between the two cell types, aside from channel density.

3.6.2 | Sustained outward rectifier (SOR)—An outwardly rectifying K^+ current (SOR) characterised most OT but not VP neurones recorded from the SON using sharp electrodes.[64,141,142] The SOR required depolarisation to approximately −60 mV, and was most easily seen making long hyperpolarising steps from approximately −50 mV, where a sag associated with its deactivation was observed at potentials nearest to −50 mV, and which was followed by a rebound depolarisation when returning to −50 mV (Figure 5). Althougth resembling the muscarine-activated K^+ current (M-current) that has been identified in SON neurones,[143] muscarine did not affect the SOR.[142]

The SOR is sensitive to millimolar concentrations of tetraethylammonium (TEA), but not to Cs^+, or 4-aminopyridine. Although showing some Ca^{2+}-dependence, the SOR is unaffected by SK or BK channel blockers. OT neurones show a longer latency spike frequency adaptation not seen in VP neurones and this may be SOR-dependent.[64] In addition, the strong presence of this persistent current at depolarised potentials might contribute to the

elevated spike threshold sometimes observed in OT relative to VP neurones.[49,64,65] Its role in the prominent rebound potential upon its deactivation and subsequent reactivation also suggests that inhibitory inputs occurring near rest could significantly modify ongoing firing patterns. Thus, the SOR may contribute to the irregular firing patterns produced by GABAergic synaptic potentials in OT neurones[44] and to the facilitation of OT bursts induced by local GABA application in lactating rats.[144]

Unfortunately, the SOR has not been observed with the same prevalence with whole-cell recordings and thus is not useful for distinguishing the two cells with this method.[44,58] However, the SOR, as well as an additional inward rectification, has been observed in OT neurones using the perforated patch method.[145] This suggests that whole cell dialysis may dilute some intracellular constituent necessary for SOR expression.

3.6.3 | Kv3-like currents—The Kv3 family of K^+ currents (Kv3.1–3.4; KCNC1–4) are high voltage, rapidly activating, TEA-sensitive (<1 mmol L^{-1}) currents that are known to influence spike repolarisation, typically in fast-firing neurones in cortex and elsewhere.[146] VP neurones more strongly express Kv3.1b subunits compared to OT neurones immunochemically, show a greater proportion of TEA-blockable (<1 mmol L^{-1}), slowly in activating current, and show a greater degree of TEA-induced spike broadening (<1 mmol L^{-1}).[73] The sensitivity of both OT and VP neurones to the Kv3.4 toxin, BDS-I, although not as great as low doses of TEA, suggests that the high voltage, rapidly inactivating component of TEA-sensitive current may be a result of the Kv3.4 subunit. To date, no corroborative evidence for the existence of this family of currents in OT or VP neurones has been made available.

3.6.4 | Na^+-dependent K^+ current—Both OT and VP neurones also exhibit a Na^+-dependent, voltage-dependent persistent K^+ current consistent with the properties of Slo2.1 (Slick) and Slo2.2 (Slack) K^+ channels, which structurally belong to the Ca^{2+-}dependent channel family, K_{Ca}4.1 (KCNT1) and K_{Ca} 4.2 (KCNT2).[147] Given their Na^+ activation and very depolarised half-activation values (approximately −4 mV), we can speculate that these channels could contribute to the small spike-activated sAHPs remaining following removal of the larger Ca^{2+}-dependent AHPs in SON neurones,[88,90] similar to that found in other neurones expressing these channels[148].

3.7 | Osmotically sensitive currents

In vivo, hyperosmotic stimuli activate both OT and VP neurones[2] and release both hormones,[149,150] although there may be differences in aspects of their release to hypertonic challenge.[149–151] It is now understood that this activation results from: (i) the direct osmosensitivity of the OT and VP neurones as demonstrated in vitro; (ii) osmoresponsive elements projecting to these neurones from regions surrounding the rostral end of the third ventricle, such as the organum vasculosum of the lamina terminalis[152,153]; and (iii) the osmosensitive astrocytes surrounding the SON neurones releasing the inhibitory transmitter taurine, which acts on glycine ionotropic receptors that pass Cl^-.[154,155]

3.7.1 | The osmoreceptor current—Work carried out with synaptically uncoupled neurones in slices, as well as isolated, dissociated neurones, has shown that SON neurones are directly osmoreceptive.[15,156–158] The basis for direct osmoreceptivity appears to be a mixed cation current carried by a stretch-inactivated type mechanoreceptor, identified as an N-terminal splice variant of the transient receptor potential vanilloid type-1 (Trpv1) channel; knockouts of Trpv1 render SON neurones insensitive to hyperosmotic activation.[159] Almost all magnocellular SON neurones appear to be osmoreceptive[160,161] and there are no data available suggesting that Trpv1 channels are differentially distributed between OT and VP neurones.

3.7.2 | Osmotically sensitive K^+ current—Liu et al[162] identified a depolarisation-activated current in isolated SON neurones that was increased by hyperosmolality. This was subsequently found to be a voltage- and Ca^{2+}-dependent K^+ current that was inhibited by muscarine and by M-current blockers, and was increased by the M-current channel opener, retigibine. Although the current was present in all of the identified OT and VP neurones, its osmosensitivity was only evident in approximately 50% of each cell type.[143] RT-PCR and immunocytochemistry data suggested that SON neurones expressed several members of the Kv7 (KCNQ) family of K^+ channels, although it is unknown whether these channels mediate this particular current. Although similar in voltage dependence and pharmacology to Kv7 type currents, other properties of this osmosensitive current, such as its inactivation, suggest differences from a classic M-current.[143] Interestingly, given its Ca^{2+} dependence, sensitivity to muscarine, and the ability of M-current drugs to modulate firing patterns, the current described by Zhang et al[143] may be involved in the sAHP.[90]

3.8 | Acid-sensing and related channels

SON neurones possess acid-sensing Na^+ channels (ASIC1 and ASIC2) that are amiloride-sensitive, voltage-independent and which, when activated with low pH, produce a large, transient inward current.[163] This current is much larger in the VP neurones studied in transgenic rats compared to OT neurones, and the expression of ASIC1 and ASIC2 channels has only been confirmed in VP neurones,[62] suggesting a role for ASICs in stimulating VP release during localised hypoxia.[163] However, OT release to this same stimulus was not examined.

ASIC channels are in the superfamily of epithelial Na^+ channels (ENaCs) and ENaCs have also been found in SON and PVN magnocellular neurones.[56] Similar to ACICs, ENaCs are sensitive to amiloride and are voltage-independent. Although α-ENaC was found in both OT and VP neurones, β- and γ-ENaCs were found only in VP neurones. Amiloride and the more specific ENaC blocker, benzamil, both reduced an inward leak current and more often hyperpolarised SON VP neurones, suggesting that ENaCs contribute to resting membrane potential to a greater degree in VP than in OT neurones. β- and γ-ENaCs neurones are up-regulated in VP neurones of rats given a high-salt diet,[164] and γ-ENaCs are up-regulated in the SON of the salt-sensitive Dahl-strain of rats.[165,166] Although changes in ENaCs in VP neurones could contribute to the development of salt-related hypertension via increases in VP release, their role in changes in VP neurone firing in these conditions is unknown. The

aldosterone receptor, known to regulate ENaC expression, is also found in SON neurones, but appears to be equally distributed in VP and OT neurones.[56,166]

3.9 | Differences in the Cl^- equilibrium potential between OT and VP neurones

Haam et al[167] found a striking difference in the Cl^- equilibrium potential between OT and VP neurones that would markedly influence GABAergic neurotransmission, as well the osmotically regulated glial transmission mediated by activation by taurine of glycine ionotropic receptors. Using perforated patch recordings (gramicidin) that maintain $[Cl^-]_i$, GFP-VP neurones from transgenic rats had a depolarised E_{Cl}^-(approximately −36 mV) compared to OT neurones (E_{Cl}^- approximately −72 mV); this rendered GABAergic synaptic potentials as excitatory near the resting potential in VP neurones. This difference correlated with a differential expression of Cl^- transporters, such that the inhibition of the K^+-Cl^- co-transporter 2 (KCC2) in VP neurones had little effect on E_{Cl}^-, whereas inhibition of the Na K Cl^- co-transporter 1 (NKCC1) produced a positive shift. On the other hand, OT neurones were unaffected by selective NKCC1 antagonism but exhibited a significant depolarising shift in E_{Cl} when KCC2 was antagonised. These results correlated with weaker immunochemical localisation of KCC2 in VP neurones compared to OT neurones. However, Choe et al,[168] also using perforated patch recording, failed to find a difference in E_{Cl}- between RFP-OT and GFP-VP labelled neurones from two different strains of transgenic rats, and found abundant KCC2 in VP neurones. Kim and colleagues also found the percentage of identified VP neurones showing GABA-mediated excitation to be low.[169]

Although it is difficult to reconcile these disparate findings, it is evident that E_{Cl}^- is labile and likely subject to different environmental conditions. Kim et al[170] found a dramatic shift in E_{Cl}^- in SON neurones after a chronic salt load, such that all neurones displayed a depolarised E_{Cl}, and found a similar depolarising shift in identified VP neurones made hypertensive with the deoxycortisone acetate-salt model.[169] Similarly, Choe et al[171] found that high-salt loading produced a depolarising shift in GABA-mediated Cl^- currents in identified VP neurones. This shift correlated with a reduction of KCC2 immunolabelling, a decreased sensitivity of E_{Cl}^- to its antagonism and an increased response to NKCC1 antagonism that resulted in GABAergic excitation of firing. Finally, a switch to GABAergic excitation also occurs with angiotensin II-induced hypertension,[172] suggesting that more than one type of chronic challenge to the VP system can produce this change.

The differential expression, or sensitivity, of Cl^- transporters in the two neurone types could have profound consequences for the efficacy of glial transmission through nonsynaptic glycine receptors,[154,155] for nonsynaptic GABA receptors likely present in the SON,[44,173] and for the synaptic plasticity observed in GABAergic synaptic density[127] and in GABAergic neurotransmission[174] in OT neurones during pregnancy and lactation. In addition, miniature inhibitory synaptic potentials are four to five times more prevalent in OT than VP neurones, at least in female rats, suggesting a basal, differential regulation of terminal GABA release that influences firing patterns.[44]

3.10 | Neurohypophysial terminal currents

The magnocellular neurosecretory system is one of few central nervous system regions where axon terminals are accessible for a variety of electrophysiological analyses and imaging.[135,175,176] Although differences between soma and terminals previously have been noted in Ca^{2+}[177] and BK[71] channels, fewer studies have focused on differences between identified OT and VP terminals, primarily as a result of technical difficulties. Such differences would be important when considering the effect different types of AP activity arriving at the terminals during bursting. For example, although bursts of action potential are known to facilitate both VP and OT release, VP release fatigues much more rapidly than does OT release during continuous axonal stimulation.[9,178] Furthermore, presynaptic opiate actions and those of other locally released transmitters, such as ATP, may depend not only on differentially distributed receptors on the terminals, but also on channel diversity.[52]

3.10.1 | Terminal Ca^{2+} channels—Both terminal types appear to exhibit L- and N-type high voltage-gated channels but differ regarding putative P/Q or R- type channels. Using an isolated terminal preparation, VP but not OT terminals were found to be immunopositive for a P/Q antibody,[179] and the K^+-induced VP release that remained in this preparation after L- and N-type blockade was blocked by the P/Q toxin, ω-AgaIVA (P/Q toxin).[117] Identified VP but not OT terminals also show a P/Q type Ca^{2+} current,[178] as identified originally in approximately 50% of unidentified terminals.[117] By contrast, the release of OT that is not dependent on L- or N- channel activity is inhibited by the R-type channel blocker, SNX-482.[114] Unlike VP terminals, OT terminals exhibit an SNX-482 blockable current,[178] a current that was previously noted in approximately 50% of unidentified terminals.[114] However, immunochemical localisation with an R-type antibody indicated its presence both OT and VP terminals, suggesting that this antibody recognised a nonfunctional channel. This is likely because SNX-482 appears to block virtually all the remaining current after L- and N-type blockade.[114,179]

3.10.2 | Terminal K^+ currents—Spike broadening as a result of K^+ current (primarily A-type) inactivation is critical to gaining the enhanced Ca^{2+} influx that underlies the facilitation of terminal hormone release.[9,135] Although there are differences in the properties of frequency-dependent facilitation for OT and VP release, and their release in response to drugs blocking either A-type and delayed rectifying K^+ currents,[180] no evidence exists at present to suggest a difference in the currents themselves in the two terminal types, nor in Ca^{2+}-dependent K^+ currents.[52]

Although not covered in the current review, the neurohypophysis contains a variety of neuroactive substances either co-localised with OT or VP, or found in non-neurosecretory axons or pituicytes that can influence hormone release.[52,181] Perhaps the best studied are the opiates dynorphin and enkephalin, ATP, adenosine, GABA and nitric oxide. The involvement of these neuromodulators in both positive- and negative-feedback during spike-dependent hormone release on hormone release could suggest a differential coupling to Ca^{2+} or K^+ channels in these terminals.[52] For example, it is clear that the elevation of nitric oxide from Ca^{2+} influx during spike trains facilitates oxytocin release via cGMP-mediated actions on BK channels,[182] although it is unknown whether a similar mechanism exits for VP

terminals. On the other hand, μ- or κ-opioid receptor activation may inhibit most Ca^{2+} channels to suppress oxytocin release, whereas μ-opioid receptor activation can actually facilitate N-channels VP neurones.[183]

4 | CONCLUSIONS

Electrophysiological studies from identified OT and VP neurones and their terminals reveal intrinsic differences that influence their firing patterns, their ability to fire repetitively and the differential release of hormone. The original studies of phasic bursting activity in VP neurones in vivo have been corroborated in vitro, with underlying mechanisms such as sDAPs and plateau potentials. Caution is needed, however, because many OT neurones display sDAPs that only rarely summate and lead to phasic bursting, such that the great majority of OT neurones fire in an irregular-to-continuous mode, much as they do in vivo when not engaged in milk ejection. Surprisingly, VP neurones exhibited larger APs, a larger underlying Na^+ current and a more stable AP threshold. When coupled with a stronger I_A delaying spiking to depolarising inputs, this suggests that VP neurones are less excitable than OT neurones. The SOR and its related rebound potential in OT neurones further suggest an additional mode of excitability in response to pattern-shaping inhibitory inputs not present in VP neurones. However, the synchronous, bursting behaviour of OT neurones exhibited during lactation appears to have no responsible intrinsic property driving this behaviour, and is likely synaptically driven in combination with the somatodendritic release of OT.[32,45,119] Thus, regardless of whether in organotypic cultures[45,119] or in hypothalamic slices,[184] the bursts of OT neurones observed in vitro that best mimic those in vivo are dependent upon both glutamatergic and oxytocinergic activity. Perhaps the most surprising difference between the two cell types is that the second messenger PIP_2 is necessary for OT neurones to express Ca^{2+}-dependent AHPs. Thus, although these AHPs are superficially similar in the two cell types, their potential for transmitter modulation is dramatically different. Considering that OT neurones show considerable up-regulation of the AHP during pregnancy and lactation part as a result of changes in the modulation SK3 channels by CK2, the obligatory role of PIP_2 in AHPs in these cells suggests that it may play a significant role in their plasticity.

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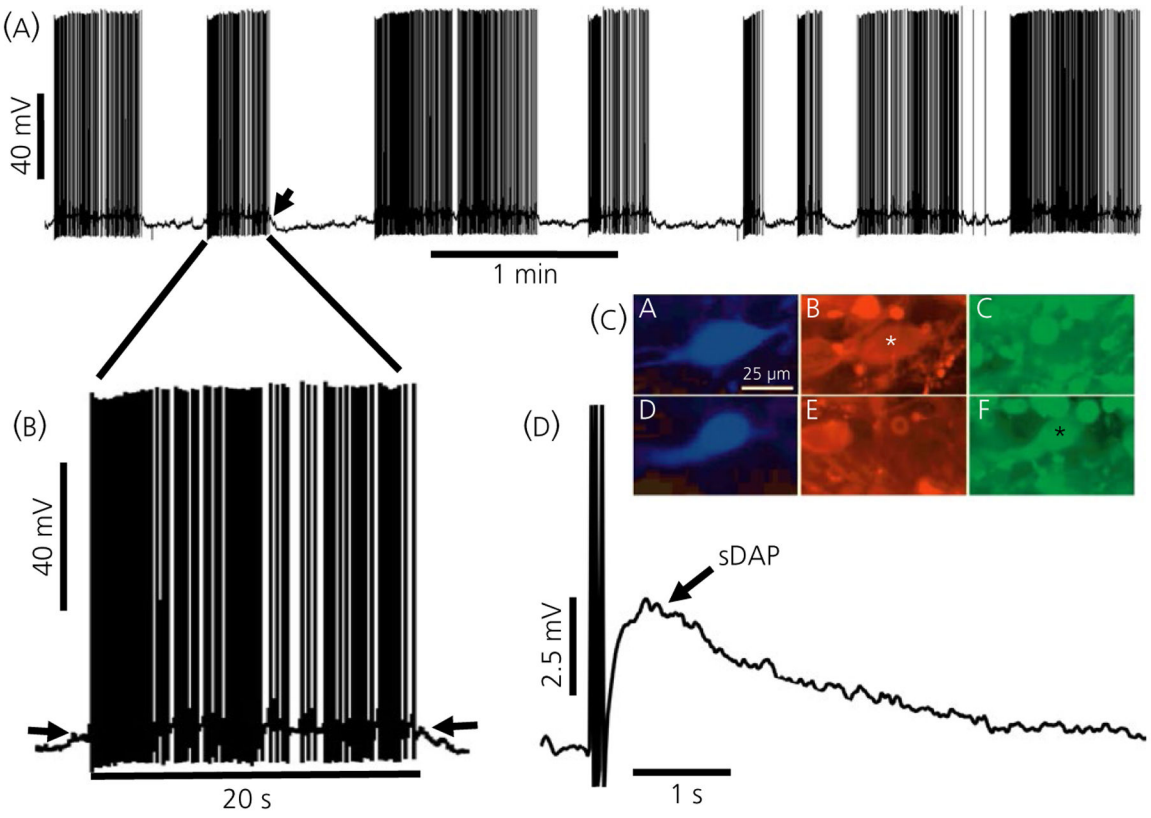

FIGURE 1.

Characteristics of vasopressin (VP) neurones. A, Phasic bursting in an immunopositive VP neurone from a hypothalamic slice. The burst indicated by the arrow is expanded in (B), where the plateau potential underlying the burst is evident (arrows). C, Examples of immunochemically identified oxytocin (OT) and VP neurones. CA, Biocytin-filled neurone OT stained with avidin-aminomethylcoumarin. CB, The neurone is positive for OT-neurophysin (NP) and negative for VP-NP (CC). CD, Biocytin-filled neurone VP stained with avidin-AMCA. CE, The neurone was negative for OT-NP and positive for VP-NP (CF). D, Slow depolarising afterpotential (sDAP) in an identified VP neurone. Parts (A), (B) and (D) are modified from Armstrong.[185] Part (C) is modified from Li et al[44]

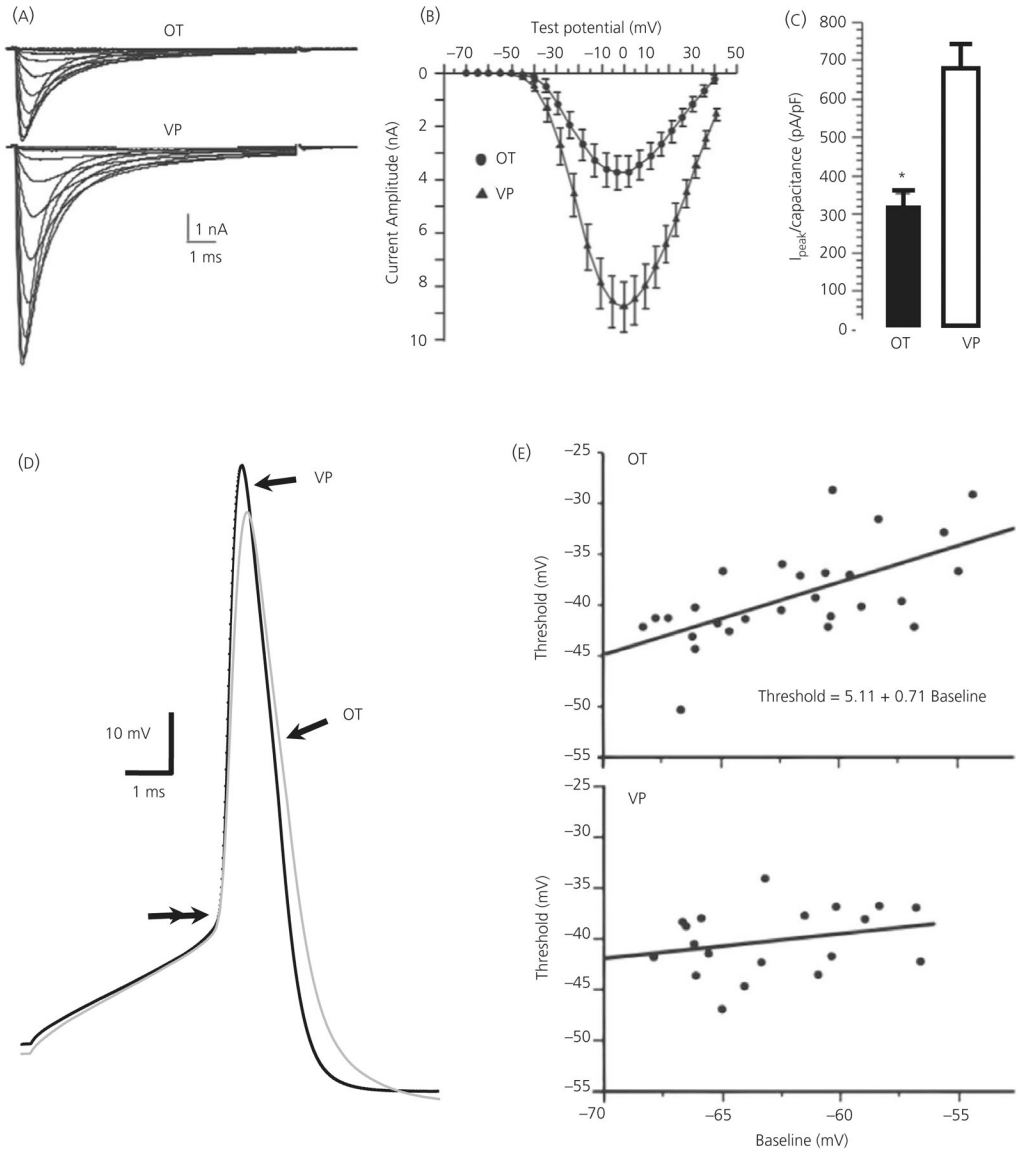

FIGURE 2.

Transient voltage-dependent Na^+ (I_{Na}^+) currents in dissociated, immunopositive vasopressin (VP) and oxytocin (OT) neurones. A, Averaged I_{Na}^+ records from 13 OT and 20 VP neurones showing voltage dependence of activation (steps to −55 through −5 mV from −90 mV). B, Plots of peak amplitude ($\bar{x}$ ± SEM) vs voltage from neurones shown in (A). C, Bar graph illustrating difference in peak current density ($\bar{x}$ ± SEM) for these OT and VP neurones, $*P \leq 0.05$. D, Action potentials (APs) from OT (grey trace) and VP neurones (black trace) aligned at threshold (double arrows). Note the slower rise of the OT compared to the AP of the VP neurone, and its smaller amplitude. E, Spike threshold plotted against baseline membrane potential (V_m) in OT and VP neurones. Top: in OT neurones, threshold was positively correlated with $V_{m.}$ Bottom: in VP neurones, no significant correlation was found. Modified from Scroggs et al[49]

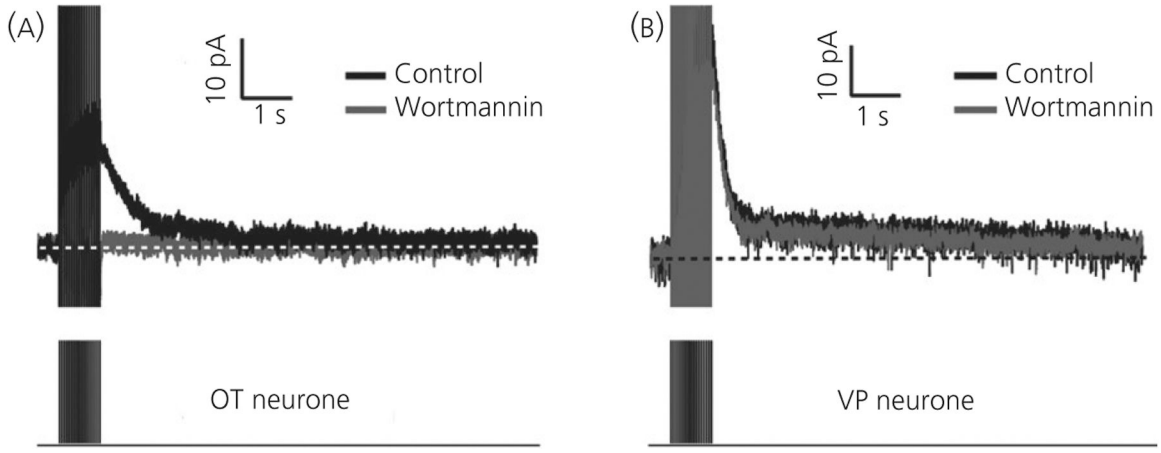

FIGURE 3.

Afterhyperpolarisation current (I_{AHP}) in immunopositive oxytocin (OT) and vasopressin (VP) neurones in slices. A, I_{AHP} in an OT neurone produced by a train of 17, 5-ms voltage pulses (−60 mV to +10 mV) at 20 Hz. The I_{AHP} is strongly inhibited by the PIP_2 blocker, wortmannin (1 μmol L^{-1}) (grey trace). B, In contrast, the I_{AHP} to the same stimulus in a VP neurone is unaffected by wortmannin. In both (A) and (B), the amplitudes of voltages pulses have been truncated. Modified from Kirchner et al[84]

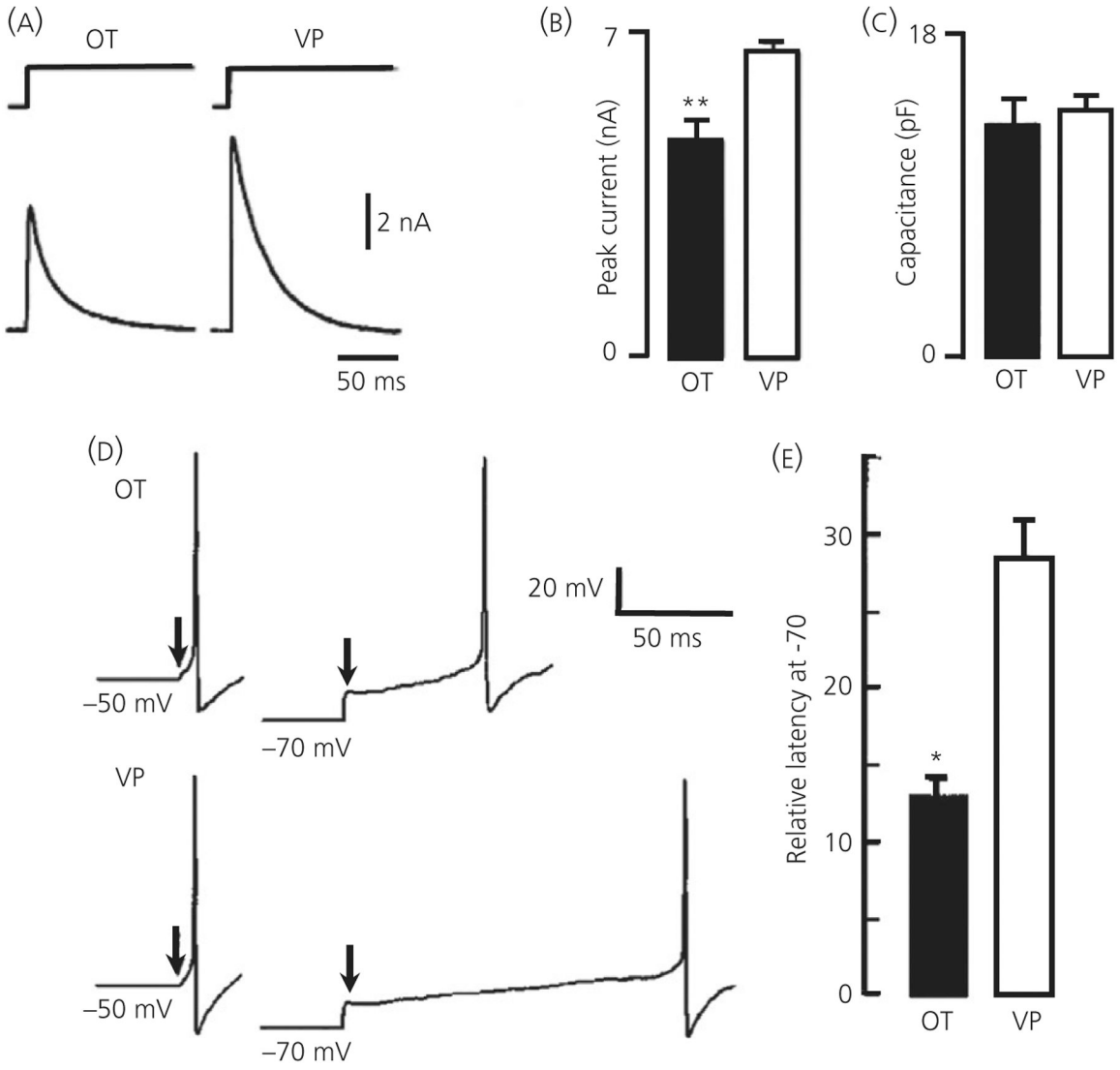

FIGURE 4.

Transient K^+ current (I_A) is greater in vasopressin (VP) neurones. A, I_A evoked with 500-ms pulse from −130 to −10 mV in an oxytocin (OT) and VP neurone under voltage clamp. B, Bar graph illustrating differences in peak current for 18 OT and 52 VP neurones ($\bar{x}$ ± SEM), ** $P \leq 0.001$. C, The whole cell capacitance is not different in these cells, indicating a larger current density in VP neurones. D, Current clamp recording showing the latency to excitation with a depolarising pulse in OT and VP neurones from holding potentials of −50 mV vs −70 mV. The time between the arrows and the action potential is the latency. E, bar histograms showing the mean ± SEM latency to excitation following release from −70 mV expressed relative to that at 50 mV. Note that the relative latency in OT neurones is <50% of that in VP neurones (* $P < 0.01$). Modified from Fisher et al[47]

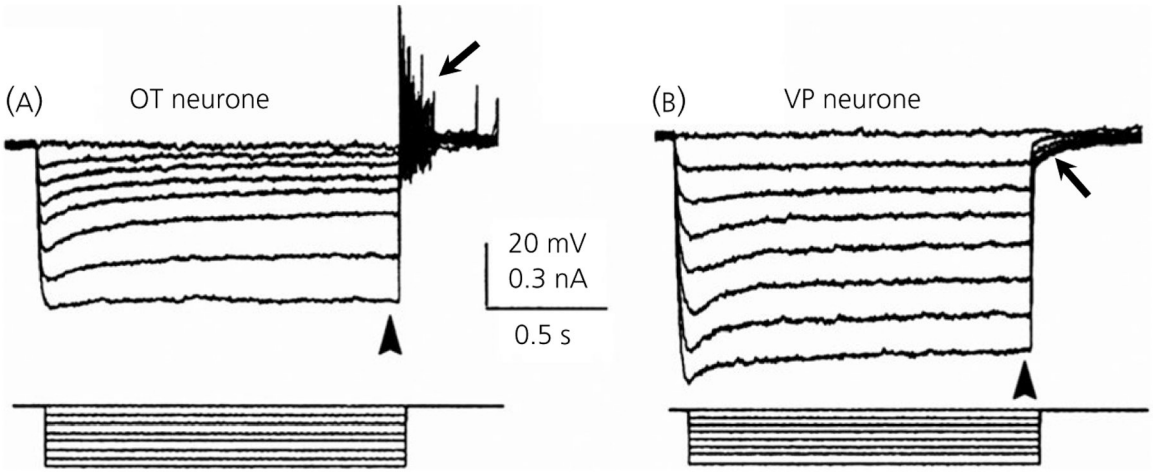

FIGURE 5.

Oxytocin (OT) neurones exhibit a sustained outward rectification (SOR) with sharp electrode recordings in slices. A, When hyperpolarised from −50 mV, a biocytin-injected, immunopositive OT neurone shows a time and voltage-dependent SOR (arrowhead) followed by a rebound depolarisation at the current offset (arrow). Note that the sag representing the SOR decreases with hyperpolarisation. B, In contrast, the vasopressin (VP) neurone hyperpolarised from −50 mV shows a slow sag that increases with hyperpolarisation consistent with some I_H, although with an otherwise almost linear I/V relationship (arrowhead). Rather than a rebound depolarisation, VP neurones show transient outward rectification at the current offset (arrow). In both (A) and (B), current steps are shown below voltage traces. Modified from Stern and Armstrong[141]