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COMMENTARY

Different species, different gap junctions?

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In the middle of the 20th century, a series of results showing an evident propagation of depolarizing and hyperpolarizing potentials between two assembled cells in the cardiac ganglion of the lobster were published for the first time in a rigorous manner (Watanabe, 1958). These findings gave impetus to the exploration of intercellular electrotonic propagation, later referred to as electrical coupling or gap junctions. These junctions are composed of pairs of hemichannels, each consisting of a hexameric complex of connexins, embedded in tightly adhered plasma membranes of neighboring cells (Fig. 1). Years later, electrical transmission was demonstrated in several neuronal junctions of vertebrates and invertebrates, but it was not until 1970 that Hinrichsen demonstrated, using intracellular microelectrodes, the existence of an electrotonic communication pathway in the mammalian central nervous system (CNS), specifically in the sensory neurons of the trigeminal mesencephalic nucleus (MesV). Previous morphological studies had shown that these tended to form clusters of two to three cells closely associated at their somata or at their somata and axon hillock (Fig. 1).

Hinrichsen (1970) recorded small, non-synaptic, fast kinetic potentials, baptized a year later by Baker and Llinás (1971) as SLD ("short latency depolarizations"). These potentials were presumably derived from other coupled cells and not from peripheral stimulation of the trigeminal nerve, since they did not observe a collision with the orthodromic action potentials evoked by current injection in the recorded cell. This detected rapid kinetics was in absolute agreement with electrical transmission (Bennet, 1966). Both studies suggested that one of the advantages of these bidirectional electric connections is the synchronization of these mesencephalic cells into a functional unit, whose effect could be the amplification of the input signal, since the discharge of an element results in the discharge of a whole group of perfectly assembled cells.

During the following decades, the primary sensory neurons of the MesV have been the object of interest, so their anatomy and functionality have been well established. At the morphological level, they differ from their homologues located in the dorsal root ganglion in being the only primary sensory neurons whose soma is found in the CNS. In most species, three classes of sensory neurons have been described in MesV: the most numerous are large spherical unipolar cells. Smaller unipolar cells, and to a lesser extent multipolar cells have also been identified (Lazarov, 2002). Functionally, they have been divided mainly into spindle afferents and periodontal afferents and a few group III muscle afferents, according to some reports. The endings of the first group innervate the neuromuscular spindles of the jaw-closing muscles while those of the second group which act as mechanoreceptors are in the periodontal ligaments, surrounding the tooth roots (Morquette et al., 2012). In addition, these neurons receive synaptic inputs onto their soma from several structures of the anterior brain, midbrain, and lower brainstem (Lazarov, 2002; Verdier et al., 2004). Therefore, firing in these particular primary afferent neurons can be triggered from their peripheral terminals or from their central synaptic inputs.

In this issue of the *Journal of General Physiology*, Dapino et al. (2023) compare the electrophysiological properties and the coupling of MesV sensory neurons of two closely related species, the rat and the mouse. The search for possible functional differences in homologous circuits stems from a previous study where it was shown that the incidence of electrical coupling in MesV is approximately three times higher in mice than in rats (Curti et al., 2012). Beyond the differences in coupling, their results also revealed differences in the electrophysiological properties of MesV neurons of both species, leading to important differences in cellular excitability and postsynaptic recruitment efficiency in MesV electrical synapses. They then searched for the membrane mechanism underlying these differences and identified D-type K^+ current (ID; Fig. 1), whose expression was higher in the mouse than in the rat, as the potential suspect.

Membrane properties of MesV coupled neurons

To address whether anatomical differences previously observed in rodent homologous circuits could lead to functional dissimilarities, Dapino et al. (2023) first compared the properties of electrical synaptic transmission between rat and mouse MesV coupled neurons. As an initial step, they stipulated the ability of the presynaptic spike to recruit postsynaptic neurons as an

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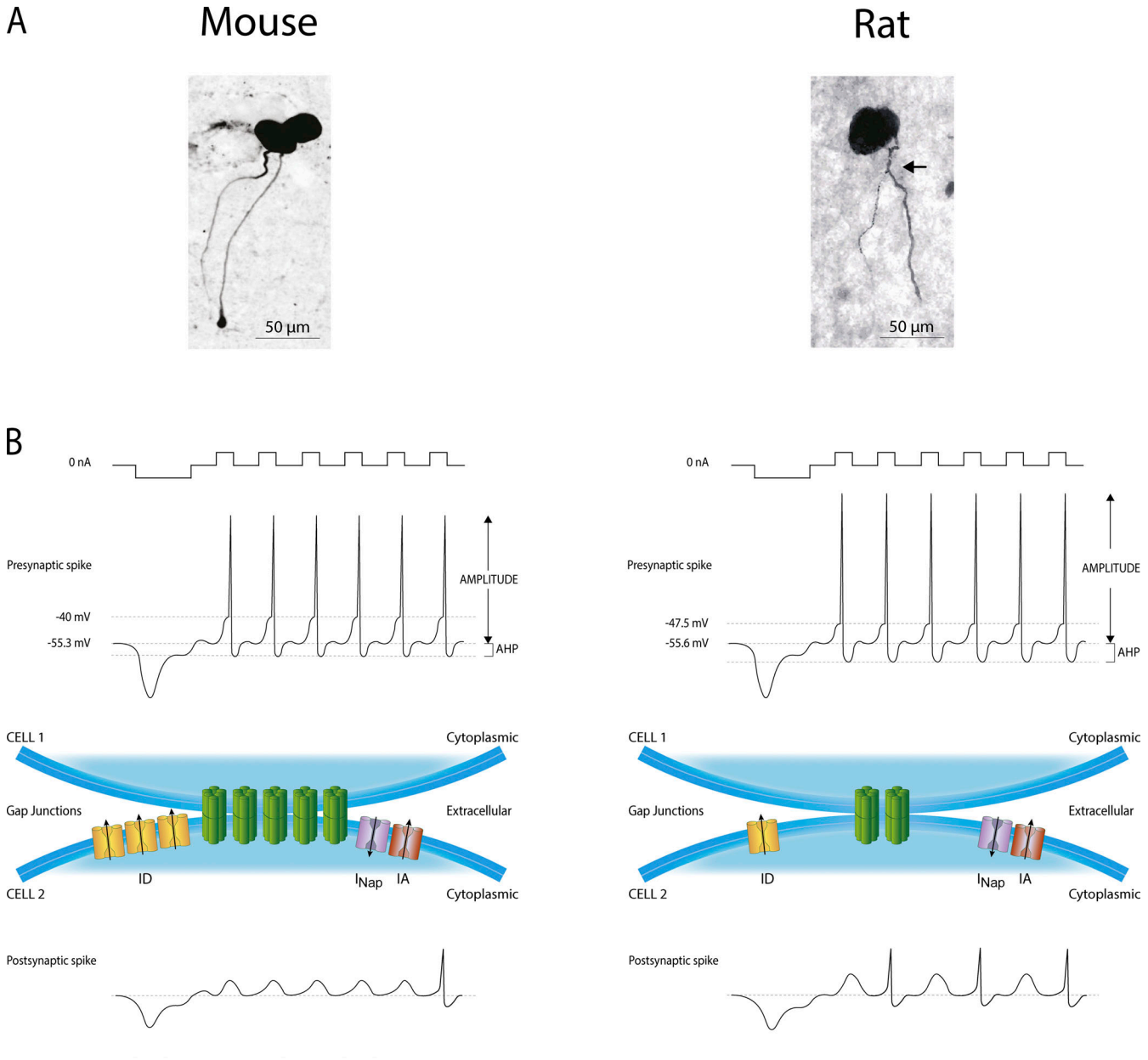

Figure 1. **Influence of membrane properties on coupling efficacy. (A)** Morphological representation of two coupled MesV neurons from mouse (left) and rat (right). Note that in addition to the connections between their somata in both species, an axon–axon communication (black arrow) can also be observed in the case of the rat. **(B)** Graphical summary of the main differences and similarities of the electrophysiological properties of the gap junctions between both species. Top: To test for coupling strength, spike transmission and postsynaptic recruitment current pulses are injected in one of the cells (presynaptic) to elicit hyperpolarization of the membrane and action potentials. Firing threshold was more depolarized in mice MesV neurons, and the amplitude of their spike and AHP was smaller. The resting membrane potential is similar in both species. Middle: Membrane mechanisms. Higher density of gap junctions in the mouse than the rat (symbolized by hexomeric connexin complexes in green). Greater K^+ type D (ID) current in the mouse (due to a higher number of K^+ channels for ID or changes in the channel conductance) and equal conductance for K^+ type A (IA) current and persistent sodium (I_{NAP}) current in both species. Bottom: Responses recorded in the second cell (postsynaptic) following current pulse injections in the first show similar coupling strength when measured passively with hyperpolarizing responses and similar amplitude of elicited spikelets, but a higher efficacy in postsynaptic recruitment in the rat.

important indicator to analyze in electrical coupling. This is consistent since this criterion supports the most common functions of electrical synapses: synchronization, lateral propagation, and forward transmission (Bennet and Zukin, 2004). Surprisingly, the results revealed a higher efficacy of postsynaptic recruitment in the rat (~50% in rats vs. ~2% in mice) despite the higher incidence of gap junctions in mice (Fig. 1) and the fact that the coupling coefficient (CC) and its two main components (input resistance and gap junction conductance) were very similar between both species. Generally, CC is defined by the relationship between the voltage change in the coupled cell and the change in the stimulated cell at steady state (Shimizu and Stopfer, 2013). When assessed with hyperpolarizing current pulses, it reflects the coupling force in a passive mode. However,

since electrical synapses can alter the signal they transmit by acting as low-pass filters that attenuate the high-frequency components of a presynaptic signal, such as action potentials, and preferentially transmit low-frequency signals, such as long hyperpolarizations (Shimizu and Stopfer, 2013), Dapino et al. (2023) assessed the spike CC. A number of differences were seen: the presynaptic spikes of mice and their after-hyperpolarization (AHP) were smaller and had a more-depolarized threshold than those of rats. Yet, CC of spikes was more efficient in mice than in rats. Rats on the other hand had larger presynaptic spike and AHP amplitude and more hyperpolarized firing threshold, but because of the attenuation produced by the low-pass filter properties of the coupling, the postsynaptic coupling potentials (spikelets) produced was of similar amplitude in the two species (Fig. 1). Therefore, the larger amplitude of the presynaptic spikes in rats cannot account for their higher efficacy of postsynaptic recruitment. All these results together led Dapino et al. (2023) to suspect that differences in postsynaptic recruitment were due to dissimilarities in membrane excitability. The results indicated that rat MesV neurons were more excitable than mouse MesV neurons, explaining their lower trigger threshold and their higher postsynaptic recruitment efficiency (Fig. 1; Dapino et al., 2023). However, since the membrane mechanism responsible for such differences was not clear, they proceeded to characterize the subthreshold K^+ and the persistent Na^+ (I_{Nap}) currents which have been associated in a number of studies to the transmission of spikes in electrical contacts, synchronization, rhythmic activity, and subthreshold oscillations of MesV neurons (Del Negro and Chandler, 1997; Wu et al., 2001; Saito et al., 2006).

$I_{4\text{-}AP}$ current in MesV coupled neurons

The intrinsic properties of the membrane of MesV neurons determine their output in response to incoming synaptic inputs or activity of their peripheral terminals. However, how these properties affect their response to input from another electrically coupled neuron has not been well characterized so far, let alone the existence of differences between species. Del Negro and Chandler (1997) found that the application of 4-aminopyridine (4-AP, voltage-dependent potassium channel blocker) unmasks a steady current of K^+ ($I_{4\text{-}AP}$), active at rest, which exerts a substantial degree of control over the excitable properties of MesV neurons. Within $I_{4\text{-}AP}$, two components have been identified in hippocampal CA3 neurons: ID fast-activating, slowly inactivating, and very sensitive to 4-AP (30 µm), and IA fast-activating, rapidly inactivating, and weakly sensitive to 4-AP (3 mM; Mitterdorfer and Bean, 2002). Dapino et al. (2023) isolated both components in their experimental protocols (voltage clamp) and compared them in coupled MesV neurons of mice and rats. Despite similarity of IA, they found the maximum conductance of ID to be higher in mouse than in rat (Fig. 1). $I_{4\text{-}AP}$ has been reported to be activated in rat MesV neurons at approximately membrane voltages of −60 mV and to be fully activated at −30 mV, with its largest activation range between −54 and −30 mV (Del Negro and Chandler, 1997). This is consistent with the results of Dapino et al. (2023), who observed a wide current window of ∼50 or 60% of maximum conductance at membrane voltages between −40 and −30 mV. The higher ID current activation observed in mice is closely correlated with the more-depolarized threshold (Fig. 1) and lower excitability of mouse MesV neurons. These results are consistent with the experiments conducted by Yang et al. (2009), who attributed to $I_{4\text{-}AP}$ an important role in determining the excitability of MesV neurons, and clearly demonstrated that the manipulation of this current can transform the excitability of this type of neurons. Other very interesting observations that attribute a significant participation to the $I_{4\text{-}AP}$ current in the modulation of spikes clearly reflected that the K^+ current modulates to a greater extent the spike initiated in the soma than the spike invading the soma from the axon. In addition, they confirmed, using immunohistochemistry, that 4-AP-sensitive K^+ channels are expressed to a greater extent in the soma than in the stem axon of MesV neurons (Saito et al., 2006). This indicates that $I_{4\text{-}AP}$ modulates the inputs that invade the soma of MesV neurons, either by synaptic connections (mentioned above) or by electrical coupling between one or more homologous neurons, as has just been clearly demonstrated by Dapino et al. (2023).

I_{Nap} current in MesV coupled neurons

It has been shown that MesV neurons produce rhythmic bursts which emerge from subthreshold membrane oscillations. Subthreshold oscillations indicate the existence of resonant properties that allow the neuron to selectively respond to different input frequencies, which enables them to actively configure the final electrical output. In addition, this resonant capacity can facilitate synchronization between electronically coupled neurons. However, all this will depend on the factors that govern the resonant properties, and in the case of MesV neurons it is the interaction between $I_{4\text{-}AP}$ current and I_{Nap} current (Wu et al., 2001). This led Dapino et al. (2023) to assess whether I_{Nap} could also contribute to the difference between species of neuronal excitability of MesV. However, the characterization of I_{Nap} in mice and rats showed no significant difference (Fig. 1). This negative result should not completely rule out the involvement of I_{Nap}, as well as other related factors, in MesV neuronal excitability differences between species. The rhythmic properties of MesV neurons are dependent on I_{Nap} which is sensitive to the extracellular concentration of Ca^{2+} ($[Ca^{2+}]_e$). In other trigeminal cells where rhythmic firing depends on I_{Nap}, rhythmic activity can hardly be elicited at $[Ca^{2+}]_e > 1.2$ mM (Brocard et al., 2006). The 2 mM $[Ca^{2+}]_e$ used by Dapino et al. (2023) is probably too high to thoroughly analyze the effect of I_{Nap} on the neuronal excitability of MesV neurons, but one can assume that it would similarly affect rats and mice MesV neurons. Another important aspect to consider is that I_{Nap}-dependent electrophysiological properties usually reach maturity in the second postnatal week. Therefore, comparing I_{Nap} at the same ages in both species and at low $[Ca^{2+}]_e$ might have yielded more information about the contribution of I_{Nap} to neuronal excitability and the efficiency of spike transmission in electrical contacts.

However, all things considered, the work by Dapino et al. (2023) has clearly demonstrated how similar circuit functions of two closely related species can be achieved through different

electrophysiological designs. In short, this work calls for caution when comparing animal physiology between species, and probably between strains of the same species.

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