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The cutaneous intrinsic visceral afferent nervous system: A new model for acupuncture analgesia

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ABSTRACT

The mechanism of acupuncture, whilst not known with certainty, has previously been considered to be stimulatory. A novel hypothesis is presented here in which C fiber tactile afferent axons bifurcate at acupuncture points and then diverge, running along acupuncture meridians, to subsequently communicate with Merkel cells. It is proposed that acupuncture disrupts the bifurcation of these axons, preventing neural transmission between Merkel cells as well as central communication with the spinal cord. Making use of the known phenomenon that acupuncture points have lower electrical resistance than adjacent skin, this hypothesis was tested using an electrical circuit model and successfully predicted the observed 10^3 reduction in skin resistance at acupuncture points. In addition to explaining acupuncture and the roles of both Merkel cells and C fiber tactile afferents, the model has greater implications for neuroscience, through the postulation of a new division of the autonomic nervous system.

1. Introduction

Acupuncture is the practice of stimulation of specific sites on the body surface for therapeutic purposes by inserting needles or similar means (such as lasers or electric currents) (Ernst, 2006). With a long and controversial history extending at least 3000 years in the Chinese literature, and possibly even longer, it is now widely used throughout the world for treatment of a range of disorders (Kaptchuk, 2002).

Yet, despite a number of systematic reviews which have demonstrated therapeutic efficacy of this technique for several pain-related conditions, the exact mechanism by which acupuncture exerts its effect is unknown (Ernst, 2006). The recent descriptions of definitive cerebral changes induced by acupuncture using modern neuroimaging techniques, including PET (Biella et al., 2001) and fMRI (Napadow et al., 2005) indicates that it exerts specific, non-placebo related, effects, and has accelerated interest in identifying its mechanism. While some clinical trials have demonstrated no significant improvement of verum acupoint acupuncture when compared with sham acupuncture (Madsen et al., 2009), this may reflect inappropriate selection of a sham control (Kennedy et al., 2008), with a recent PET neuroimaging study documenting definitive cerebral neurochemical differences between verum and sham acupuncture (Harris et al., 2009).

Whilst Traditional Chinese Medicine (TCM) ascribes acupuncture's effect to correction of an imbalance between *Yin* and *Yang*, restoring normal energy flow (*qi*), via stimulation of points on the skin surface which correspond to lines of flow of *qi*—the meridians (Ernst et al., 2001), there has been little scientific evidence to support this (Ernst, 2006).

Instead, modern scientific theories are based upon one of: (i) stimulation of Aβ fibres in the skin and muscle conducting impulses to the spinal grey matter, inhibiting painful stimuli from the periphery and reducing pain perception, (ii) activation of encephalin-containing interneurones in the substantia gelatinosa of the spinal grey matter resulting in inhibition of the conduction of pain signals to the brain, (iii) release of beta-endorphin and met-encephalin in the brain, (iv) activation of descending pain control systems in the mid-brain, and (v) modulatory effects on the central pain network in the hypothalamus and the limbic system (Ceniceros and Brown, 1998). The long known finding that acupuncture points have altered electrical resistance (Ahn et al., 2005) has also led to a novel theory invoking signaling along a fibroblast network (Langevin et al., 2006). Indeed, evidence for a connective tissue network was originally suggested by Kim (1963) on the basis of the demonstration of connective tissue channels, or Bonghan's Ducts, using scintigraphic imaging of P^{32},

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subsequently confirmed with Tc^{99m} imaging (Kovacs et al., 2000) and histological studies (Lee et al., 2006). If, indeed, such a network exists, its structure and role have yet to be defined, although there is some evidence that these channels contain neurotransmitters (Kovacs et al., 1992).

Whilst Melzack and Wall's Gate Control Theory of Pain (Melzack and Wall, 1965) has been invoked as the basis for acupuncture's pain relief (Melzack, 1981), there is little evidence in the literature detailing the exact mechanism. Indeed, there appears to be a reduction in both nerve fibers (Carlsson et al., 2006; Wick et al., 2007) and cutaneous neural receptors (Monteiro-Riviere et al., 1981) at acupuncture sites. Yet, cells derived from the neural crest, containing neurotransmitters, are found in the deep epidermis and dermis—Merkel cells (Lucarz and Brand, 2007). These are excitable cells that are capable of glutamate and neuropeptide release at synaptic junctions (Haeberle et al., 2004), and also appear to be involved in nociception, given that they contain an endogenous opioid-like substance, dynorphin A (Weihe et al., 1998). Whilst a number of hypotheses, including mechanoreception, nociception and neuromodulation, have been suggested for their function, the exact role of Merkel cells remains unknown (Boulais and Misery, 2007).

2. A new anatomical–physiological model

Current theories that explain acupuncture on the basis of Melzack and Wall's (1965) gate control theory of pain, suggest that the insertion of needles into the skin results in stimulation of Aβ fibers, resulting in inhibition of sensory neurons, "closing the gate" (Fig. 1). Whilst this theory successfully explains transcutaneous electrical nerve stimulation (TENS) and has been applied to the development of implantable spinal stimulators (Fann et al., 2007), it has not adequately explained how acupuncture exerts its effect, given that acupuncture points may have no anatomical relationship, i.e. may be at considerable distance to sites of pain origin. An alternative theory might invoke the nociceptive side of the gate, but would need to explain how acupuncture—often at a significant distance from the site of pain—could exert a distant neurophysiological influence. The C fiber afferents are known to be widely distributed in the skin and are likely to have a role in pain transmission (Wessberg et al., 2003). This has raised interest in these fibers as a conduit for the acupuncture effect, but an exact mechanism has yet to be enunciated (Campbell, 2006). Given that these fibers carry nociceptive information, any theory explaining acupuncture in terms of these fibers would need to define how the insertion of an acupuncture needle that stimulates a pain-carrying C fiber at considerable distance, would cause pain reduction at another site.

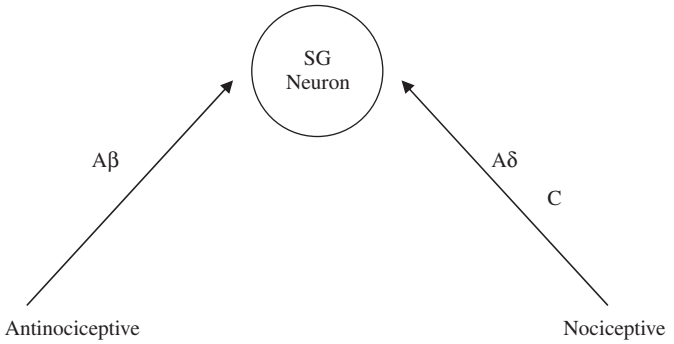

Fig. 1. The gating theory of pain schematic diagram of afferent input to substantia gelatinosa (SG) neuron, based upon Melzack and Wall's Gate Control Theory of Pain (1965).

Yet, no previous model has considered the simple fact that, rather than stimulating, the insertion of an acupuncture needle might be disruptive.

For this to occur, there would need to be a network which normally transmits excitatory impulses to neurons at some distance from the site of pain perception. This network would need to continuously fire at a rate which—though exciting local pain-sensing neurons—was balanced by the ongoing firing of Aβ fibers. If the nociceptive limb—carried by C fibers—was disrupted by an acupuncture needle—the balance of activity would result in reduced stimulation of the pain-sensing neuron, closing the gate and causing pain relief. An anatomical model which would explain this is displayed in Fig. 2. In this scheme, a visceral sensory afferent neuron, with cell body in a dorsal root ganglion, gives rise to an axon which extends to a subepidermal location, and then bifurcates in a "T" shape. Unmyelinated cutaneous axons that bifurcate in this manner have been previously described, and are known to demonstrate both antidromic and orthodromic conduction (Morris and Gibbins, 1997). What would these diverging axonal divisions synapse with? It is known that isolated Merkel cells are closely related to free nerve endings of C fibers (Zhang et al., 2002), and that these cells are capable of neurotransmitter release (Haeberle et al., 2004). If Merkel cells were the target of this T bifurcating axon, this would explain their capacity for neurotransmitter release and suggest a possible function—transmission of low grade nociceptive stimulation, not necessarily induced by noxious stimuli. The firing of one Merkel cell would result in transmission in 2 directions—tangential transmission to the next Merkel cell via the horizontal limb of the T axon—and centripetal transmission via the vertical limb to the sensory neuron's cell body. This invokes a dual pathway for low grade sensory enhancing neuro-transmission along the nociceptive arm of Melzack and Wall's Gate. The insertion of an acupuncture needle into the T bifurcation (Fig. 3) would disrupt both pathways. The "T" bifurcation would be expected to occur at the dermo-epidermal junction, deep to the location of an acupuncture point, explaining the paucity of Merkel cells at these sites (Monteiro-Riviere et al., 1981). The T bifurcating axon would also be expected to be lined by an endoneurial sheath—including a Schwann cell, as recently identified by Tschachler et al. (2004)—which could explain the previously described—and unexplained—Bonghan's ducts (Lee et al., 2006). Injection of a radionuclide into an acupuncture point (according to Fig. 3) could result in uptake by the axon and longitudinal transmission along the line of an acupuncture meridian to its termination at a Merkel cell. Axonal transport along a meridian appears to be inconsistent with Kovacs et al., who first demonstrated Tc^{99m} transport along a meridian following acupuncture point injection, stating: "Migration of Tc^{99m} through nerve fibers can be excluded since the radioactive path does not coincide with any anatomical structure of this type nor does the diffusion speed suggest this either. Moreover, no evidence is available to show that Tc^{99m} has affinity for nerve tissues" (Kovacs et al., 1992). However, a subepidermal single axon would not be expected to have been formally described in previous anatomical atlases, and transmission rates through a single C fiber would be expected to be far slower than through typical peripheral nerves (Wessberg et al., 2003). Finally, technetium is one of only three Group VIIb elements in the periodic Table, another of which is manganese, known to be actively taken up by peripheral axons and transmitted centripetally (Watanabe et al., 2002). With similar properties to Mn, it would not be surprising if Tc were also capable of axonal uptake and transport. The endoneurial sheath of the T bifurcating axon would also be consistent with the "threads" described by Lee et al. (2006), thought to be the anatomical representation of Bonghan's ducts and contain both

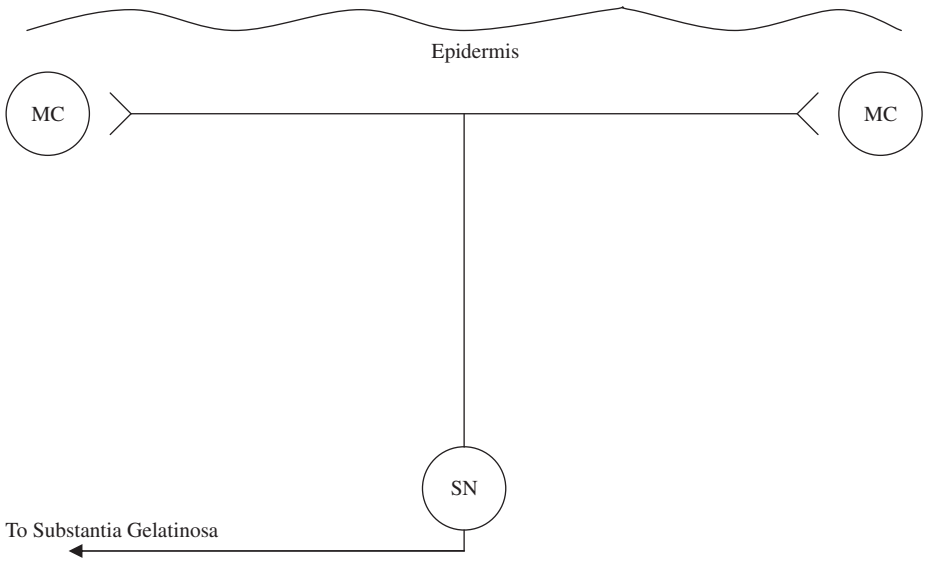

Fig. 2. T bifurcation of superficial axon. Postulated structure of visceral sensory neuron (SN), with superficial axon bifurcating to form a subepidermal "T" shape, thus creating a synaptic connection between 2 Merkel cells (MC). Note how the site of the T bifurcation is devoid of Merkel cells.

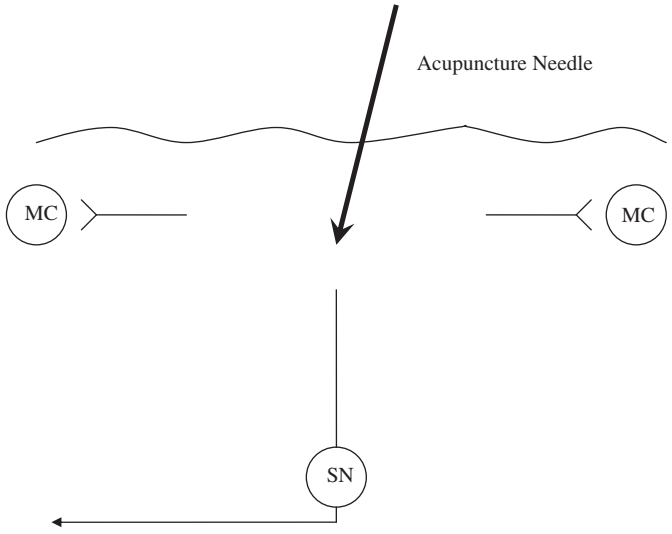

Fig. 3. Acupuncture disruption of T bifurcation. Insertion of an acupuncture needle disrupts both tangential synaptic transmission between Merkel cells and centripetal transmission to the sensory neuron.

neurotransmitters and large quantities of hyaluronic acid, also explicable if they contained axons (Alvarado and Castejon, 1984).

3. Electrical circuit modeling of an acupuncture point

Acupuncture points have long been known to demonstrate significantly lower electrical resistance than adjacent skin (Hyvärinen and Karlsson, 1977). In clinical practice, these points are located using a modified ohmmeter, with one lead placed on the point, and the second lead placed on a distant skin location or held in the hand. An electrical circuit model that is compatible with this approach, based upon the preceding theory, is displayed in Fig. 4. In this figure (b), the left-sided lead of the ohmmeter has been connected to a "T bifurcation" of a circuit, where the two limbs of the T diverge, each crossing resistances (labeled "*x*") to continue, respectively, either to earth (left-sided limb) or, via a capacitor ("*y*", simulating a simulated synapse with a Merkel cell),

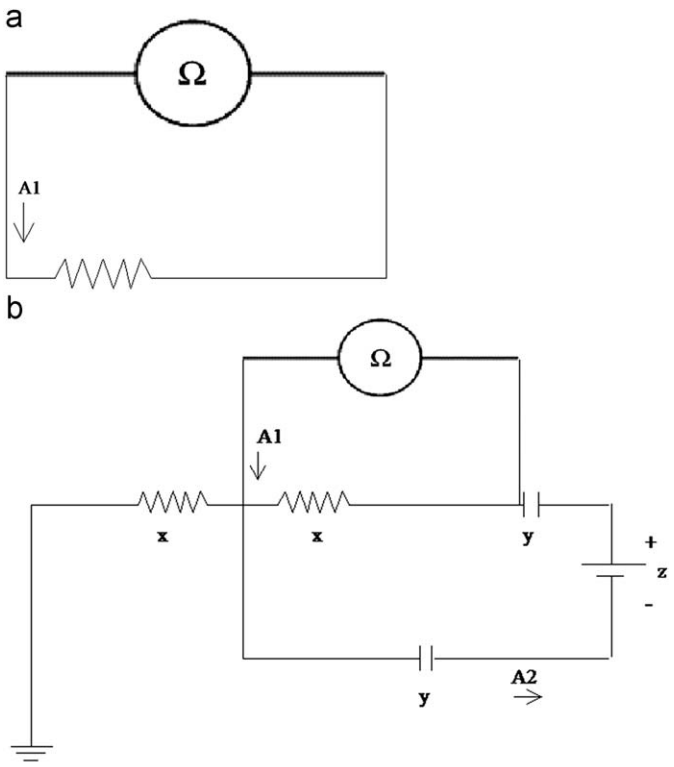

Fig. 4. Electrical circuit modeling of an acupuncture point. (a) An electrical circuit model of an acupuncture point locator (ohmmeter at top of figure) measuring skin resistance. A1 is the current running through the ohmmeter. (b) An electrical circuit model of the ohmmeter connecting in parallel with a closed axonal circuit. The vertical wire (between two resistors, *x*), represents a T-bifurcating axon, with the right limb connecting, via a capacitor, *y* (representing a Merkel cell) to a distant axon. The axonal circuit current, A2, is powered by a voltage, *z*.

to a current source ("*z*"). This simulation of a neuronal circuit is compatible with previous descriptions (Goldfinger, 2000) and incorporates the model described above. The right-sided lead of the ohmmeter is connected to the right limb of the "T bifurcation".

Using Ohm's Law, skin devoid of acupuncture points and meridians (Fig. 4a) should have a resistance of

$$R_{\text{Skin}} = V_{\text{Ohm}}/A1$$

where V_{Ohm} represents the ohmmeter's voltage, and $A1$ represents the current generated by the ohmmeter.

Based on the "T bifurcation" theory and Fig. 4(b), an acupuncture point's resistance would be

$$R_{\text{Acupoint}} = V_{\text{Ohm}}/(A1+A2)$$

where $A2$ is the current generated in the closed circuit between sequential T-bifurcating axons, Merkel cells and central synapses in the Substantia Gelatinosa. This is based upon the fact that the ohmmeter has been connected in parallel with the cutaneous circuit, so that the total current in the circuit is the sum of the currents in the individual circuits (Bakshi and Bakshi, 2008).

Now, if: $A2 \gg A1$, then $A1+A2$ can be approximated by $A2$, so that

$$R_{\text{Acupoint}} \sim V_{\text{Ohm}}/A2$$

Hence, the ratio between the resistance of an acupuncture point and that of skin elsewhere can be approximated as

$$R_{\text{Acupoint}}/R_{\text{Skin}} \sim A1/A2$$

Typically, skin resistance (non-acupoints) is around 3 MΩ (Hyvärinen and Karlsson, 1977), so that an ohmmeter (and acupoint locator) operating at 9 V should measure a current of 3 μA. A typical value for axonal current ($A2$) is around 10 mA (Goldfinger, 2000), so that this model predicts a 10^3 reduction in resistance at acupuncture points. How does this compare with clinical measurements? Acupoint resistances are around 10 kΩ, compared to 3 MΩ for surrounding skin (Hyvärinen and Karlsson, 1977), a drop in resistance by a factor of 10^3 at acupuncture points, in line with the result predicted by this theory.

Why is the ohmmeter (or acupoint locator) not short-circuited in this situation? Under usual operating conditions, the parallel connection of an ohmmeter to a closed circuit triggers the meter's internal circuit-breaker to avoid its destruction by heat created from the additional power transferred from the current source (Nilsson, 1968). This occurs through a voltage detection mechanism in the ohmmeter. However, axon voltages are only in the order of 100 mV, which is well below the meter's detection threshold (operating via a 6 or 9 V battery), and the total additional power (generating heat) from the axonal circuit is

$$\text{Voltage} \times \text{Current} = \text{Power}$$

$$100\,\text{mV} \times 10\,\text{mA} = 1\,\text{mW}$$

which is insignificant in terms of heat generation.

4. Implications of this model: the cutaneous intrinsic visceral afferent nervous system

In addition to offering a plausible explanation for the mechanism by which acupuncture exerts its analgesic effect (through disruption of subepidermal afferent axon bifurcations), this model also answers two fundamental neurobiological questions: what do Merkel cells do, and what is the role of C tactile afferent fibers?

The function of Merkel cells has, due to their probable neural crest origin, long been the subject of conjecture, because of: (1) their ability to produce neurotransmitters (Haeberle et al., 2004); (2) their rhythmic pacemaker properties, typically observed in neurons and other excitable cells (Luo et al., 2007); and (3) the fact that their ablation with quinacrine results in no change in sensory function (Mills and Diamond, 1995). Some authors have suggested that Merkel cells are "neuro-modulators", affecting the excitability of afferent nerve endings by releasing neurotransmitters (Hartschuch et al., 1983) and the presence of voltage dependent K^+ and Ca^{2+} channels in these cells suggests similar excitable properties to neurons (Yamashita et al., 1992).

C fiber tactile afferents are unmyelinated autonomic axons that are known to be widely distributed in the skin of mammals, and their afferent fields have been mapped (Wessberg et al., 2003). Their role is not known for certain, although it has been proposed that they are involved in "emotional" touch, as opposed to the discriminatory touch role of somatic afferents (Olausson et al., 2002). These axons appear to run in a linear, branching framework, and only in hairy skin (Liu et al., 2007), a pattern that is very similar to a previously described system of cutaneous linear sympathetic nerves, which appeared to run along rat acupuncture meridians (Liu et al., 2005). Functional studies have also demonstrated that these axons have a close relationship with (and have terminals in close apposition to) Merkel cells (Zhang et al., 2002). Indeed, dynorphin A, known to be located within Merkel cells (Weihe et al., 1998), has been shown to increase the release of substance P, a neurotransmitter associated with nociception, from C fiber afferent terminals (Arcaya et al., 1999). What of glabrous skin, where C fibers are not present, but meridians and acupoints are well known? Non-peptidergic unmyelinated afferents with purinergic receptors, which are known to have an important role in nociception (Burnstock and Wood, 1996), are widely distributed in glabrous skin (Taylor et al., 2009), and could have a similar role to C fibers for non-hairy skin meridians such as the pericardial meridian in the palm.

If, indeed, Merkel cells communicated directly via C fiber tactile afferents axons—albeit that these axons might be connected to cell bodies in dorsal root ganglia—then this would not be dissimilar to the enteric nervous system, where an independent system of nerves functions autonomously, but maintains a communication with the central nervous system (Furness, 2006). The extensive network of tangential cutaneous axons, coupled with their communications with the large numbers of Merkel cells might then be considered a new division of the autonomic nervous system-the cutaneous intrinsic visceral afferent nervous system. Why would this system be present and what is its role in health and disease? Recent studies in patients with chronic pain have demonstrated heightened arousal, based on measurement of reticular activating system (RAS) generated sleep state-dependent P50 midlatency auditory evoked potentials (Fann et al., 2005). In addition, acupuncture has been shown, in a separate study, to decrease P50 potentials, i.e. acupuncture may result in reduction in RAS arousal (Bray et al., 2005). The sensory inputs into the RAS are extremely complex, but it is clear that living organisms maintain a constant state of arousal. Indeed, most neuroscientists accept that the brain generates and maintains wakefulness regardless of sensory stimuli during the active part of the day (Jones, 2003). What if an intrinsic stimulant for the RAS came from constant low level discharge of the autonomic nervous system—along the lines of the system suggested for the cutaneous intrinsic visceral afferent nervous system? It is well known that hyperalgesia can be induced by sleep deprivation (Onen et al., 2001), and that this is not due to a direct opiodergic neurotransmitter effect (Nascimento et al., 2007). It may well be that low level activity in the cutaneous intrinsic visceral afferent nervous system, when balanced by antinociceptive Aβ activity, is perceived as arousal, whilst an imbalance, due to e.g. sleep deprivation, is perceived as heightened pain awareness. The neuroimaging finding that acupuncture results in increased brainstem and hypothalamic activity (Napadow et al., 2005) is also in concordance with a role in relation to reticular activation and inhibition. The cutaneous intrinsic visceral afferent nervous

system would assist in the maintenance of living organisms' constant state of arousal, enhancing preparadness for a "fight and flight" response.

5. Conclusions

Despite wide clinical usage, the only physical findings definitely associated with acupuncture in living subjects have been with functional brain imaging (Napadow et al., 2005); and the measurement of lower electrical skin resistance at acupoints (Ahn et al., 2005). The Merkel unmyelinated afferent network hypothesis presented here, in which acupuncture disrupts the bifurcation of a C fiber tactile afferent axon, preventing neural transmission between Merkel cells, and supported by the accompanying electrical circuit analysis described above, offers the first rational explanation for the electrical properties and functional behavior of acupoints. Using an electrical circuit model, the hypothesis predicted the observed 10^3 reduction in skin electrical resistance at acupuncture points.

What steps are required to confirm this theory? Firstly, while acupoints have previously been analysed histologically (Li et al., 2004), detailed analysis of axonal staining of the dermal-epidermal junction (with e.g. PgP9.5) has not, and it is this investigation which offers the best chance of demonstrating the T-bifurcating axons, and, if followed tangentially, their termination at Merkel cells. Secondly, electrophysiological techniques—along the lines of Zhang et al.'s (2008) recent work—both before and after administration of quinacrine (Mills and Diamond, 1995)—could be used to confirm the role of Merkel cells as "intermediate neurons" in the proposed network.

In addition to explaining acupuncture, this model has far-reaching implications for neuroscience, through the postulation of a new division of the nervous system. The autonomic nervous system (ANS) has previously been considered to have three divisions: the sympathetic, parasympathetic and enteric nervous systems. If confirmed, the cutaneous intrinsic visceral afferent nervous system would represent a fourth division of the ANS, which is not necessarily surprising given the common ectodermal origin of skin and central nervous system.

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