

Chapter 8

Synaptic Plasticity and Synchrony in the Anterior Cingulate Cortex Circuitry: A Neural Network Approach to Causality of Chronic Visceral Pain and Associated Cognitive Deficits



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Abbreviations

ACC	Anterior cingulate cortex
AP5	Aminophosphonopentanoic acid
AUC	Area under the curve
ANOVA	Analysis of variance
BLA	Basolateral amygdala
CRD	Colorectal distension
DNQX	Cyanonitroquinoxaline dione
EA	Egg albumin
GFP	Green fluorescent protein
IBS	Irritable bowel syndrome
LFP	Local field potential
LTP	Long-term potentiation
MT	Medial thalamus
NMDA	N-methyl-D-aspartate
NVP-AAM077	[(R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-methyl]-phosphonic acid
pACC	Perigenual anterior cingulate cortex
RNAi	RNA interference
siRNA	Small interfering RNA

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RGT	Rat gambling task
SFC	Spike-field coherence
STA	Spike-triggered average
TBS	Theta burst stimulation
VH	Viscerally hypersensitive
VMR	Visceromotor response

8.1 Brain Targets for Visceral Pain “Memory” Process in the Visceral Hypersensitive State

The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. This definition is based on the concept of pain as a perception rather than as a purely sensory modality, and takes into account the fact that for pain to be consciously experienced, cognitive processing is required.

Visceral hypersensitivity is common among patients with irritable bowel syndrome (IBS). Patients with IBS may be presented with persistent severe pain, yet there are no clearly identifiable clinical or radiographic abnormalities. The mechanisms underpinning the transition from acute into chronic pain, such as prolonged “functional visceral pain” remain unclear (Mayer et al. 2000). Abnormalities that up-regulate signal intensity anywhere in the afferent system can induce hypersensitivity and pain. It has been shown that peripheral sensitization results from an increase in the sensitivity and excitability of the afferent nerve itself and/or the dorsal horn of the spinal cord (Gebhart 2000). The brain interprets and influences the perception of pain-sensation signals transmitted from the gut. Recently, functional magnetic resonance imaging (fMRI) studies are beginning to address the possible neural mechanisms of hyperalgesia in patients with IBS. The anterior cingulate cortex (ACC) is a major cortical component of the limbic loop system, and its functional relationship to emotional and motivational responses has been well described (Vogt et al. 2003). Studies of both humans and animals consistently suggest that the ACC and its related areas are important for processing pain perception (Mayer et al. 2000; Vogt and Robert 1993). Patients with IBS show enhanced activation of the dorsal ACC, and a reduction of this pattern is associated with a reduction in IBS symptoms (Vogt and Robert 1993; Fan et al. 2009). These findings suggest that the emotional and sensory components of the brain pain experience system in IBS are dysfunctional. fMRI is blood oxygenation level-dependent (BOLD) imaging, measuring neuronal activity indirectly via its assumed haemodynamic correlate. BOLD fMRI reflects changes in cerebral blood volume, cerebral blood flow and oxygen consumption. Inconsistencies exist in BOLD fMRI studies (Mayer et al. 2000; Mertz et al. 2000; Silverman et al. 1997; Sidhu et al. 2004). Such variation emphasizes the need to learn how to interpret the BOLD fMRI signal in terms of the neuronal synaptic activity and action potentials in the brain (Arthurs and Boniface

2002). However, the literature is conspicuously devoid of a definitive study that demonstrates the neural electrophysiological activity of the ACC during processing of visceral nociceptive stimulation.

8.1.1 Viscerally Hypersensitive Rat Model

Visceral hypersensitivity in rats was induced by colonic anaphylaxis (Gao et al. 2006; Cao et al. 2008). The rats were injected intraperitoneally with 10 µg egg albumin (EA antigen) and 10 mg aluminum hydroxide (adjuvant) in 1 ml saline. From the third day to the fifth day after antigen injection, the rats were given a colonic perfusion with antigen solution followed by 30 mmHg colorectal anaphylaxis for 30 s repeated 5 times with 3-min intervals.

Food allergens may be important in IBS. Previous studies have shown that luminal antigenic challenge in the sensitized rat intestine resulted in induction of contractile activity and diarrhea (Scott et al. 1998; Nanda et al. 1989). Additionally, a clinical trial has shown that patients with IBS improved with dietary exclusions (Nanda et al. 1989). Intestinal anaphylaxis enhances the activity of the intestinal mesenteric nerve (Nozdrachev et al. 1999) and triggers neuronal activations in the nucleus of the solitary tract (NTS) (Scott et al. 1998). In the intestine, the anaphylactic response is characterized by IgE antibody mediated mast cell degranulation. Studies in animals have provided evidence that mast cell activation triggers visceral hypersensitivity and gastrointestinal motor dysfunction. A recent study indicated that colonic mast cell infiltration and mediator release in proximity to mucosal innervation may contribute to abdominal pain perception in IBS patients (Giovanni et al. 2004). Thus, mast cells could be involved in the disturbed sensory-motor function of IBS. Hence the EA rat model of visceral hypersensitivity with chronic presence of mast cells may be a suitable model to study visceral hypersensitivity. However, this VH rat model has certain limitations. For example, studies indicate that psychosocial trauma and psychological distress play important roles in the onset and modulation of IBS symptoms. Nevertheless, it is well recognized that different factors may be involved in the pathogenesis of IBS.

8.1.2 Enhanced ACC Nociceptive Transmission in Viscerally Hypersensitive Rats

Direct electrophysiological evidence of the sensitization of ACC neurons in viscerally hypersensitive rats has been provided (Gao et al. 2006; Wu et al. 2008). Recording single ACC (Cg1, Cg2 and prelimbic cortex) neuronal activities in response to colorectal distension (CRD) showed that viscerally hypersensitive rats have enhanced ACC spontaneous activity, decreased CRD pressure threshold to

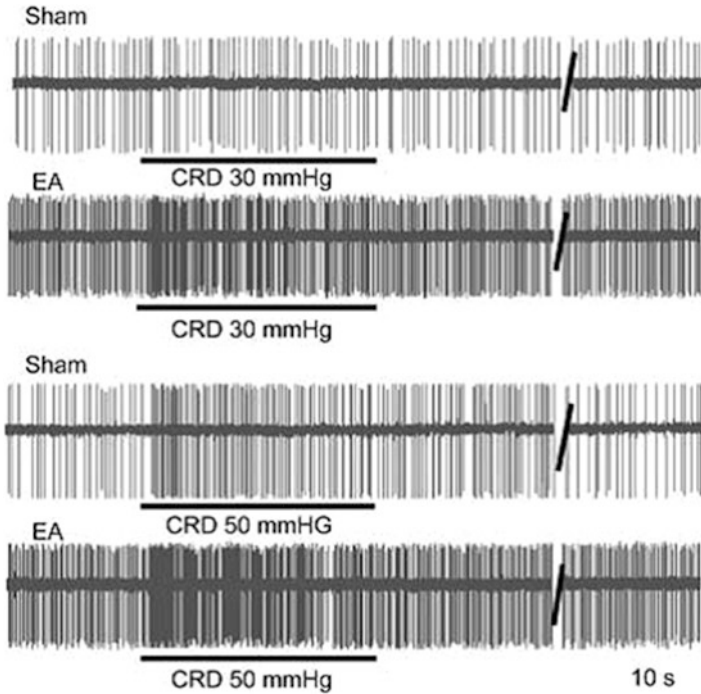


Fig. 8.1 Recordings of colorectal distension (CRD)-excited anterior cingulate cortex (ACC) neurons in response to CRD (30 and 50 mmHg) in sham-treated and viscerally hypersensitive (EA) rats. In sham-treated rats, there was a slight increase in response to 50 mmHg CRD, whereas both low- and high-pressure distension evoked increased responses in the rats sensitized with chicken egg albumin (Adapted from Gao et al., *J Physiol (Lond)* 570(1):169–184, 2006. With permission.)

stimulate ACC neurons, and increased ACC response magnitude. The ACC neurons of controls failed to respond to 10 or 30 mmHg CRD. In contrast, CRD (10, 30 and 50 mmHg) markedly increased ACC neuronal responses of EA rats (Fig. 8.1). CRD produced greater pressure-dependent increases in ACC spike firing rates in VH rats compared with controls. There are significant increases in the numbers of CRD-excited ACC neurons in the VH rats compared with normal rats (Gao et al. 2006). It appears that colorectal anaphylaxis resulted in sensitization of high-threshold receptors and brought into play previously unresponsive silent nociceptors. Splanchnicectomy combined with pelvic nerve section abolished ACC responses to CRD in VH rats. However, acute nerve section failed to prevent the enhancement of ACC spontaneous firings in the sensitized rats, suggesting that the subspinal peripheral ongoing activity is not required to maintain a higher level of ACC spontaneous activity in visceral hypersensitive rats, a phenomenon that probably originates centrally rather than peripherally (Gao et al. 2006). This electrophysiological evidence suggests that the level of activation in the ACC evoked by noxious CRD in VH state is a determinant in emotional and behavioral reactions to pain.

Visceral stimulation activates a more anterior part of the ACC compared with cutaneous stimulation (Silverman et al. 1997; Lotze et al. 2001). Previous studies revealed that most caudal ACC neurons did not respond to CRD (Gao et al. 2006). fMRI scanning has shown spatially distinct ACC activation during visceral and cutaneous noxious stimulation (Verne et al. 2001). We examined the response of CRD-excited ACC neurons to transcutaneous electrical stimulation (TCES) of the hind paw. One group of CRD-excited ACC neurons was activated exclusively by CRD stimulation. These neurons failed to respond to TCES, which suggests involvement of a population of rostral ACC neurons in visceral nociception and a possible discriminative aspect of visceral nociception. The other group of rostral ACC neurons responded to both CRD and TCES. We showed that a group of ACC neurons in VH rats exhibited enhanced CRD-induced activities. However, neuronal responses evoked by cutaneous noxious heat stimulation did not change significantly (Gao et al. 2006). This is the first demonstration that a population of rostral ACC neurons is capable of discriminative coding for hypersensitivity, specifically, visceral hypersensitivity. It should be noted that the criteria used to define rostral and caudal ACC in current studies do not correspond to the terminology of ACC regions in primates and humans (Vagt 2005).

8.1.3 N-Methyl-D-Aspartate (NMDA) Receptor Mediate ACC Synaptic Responses After the Induction of Visceral Hypersensitivity

Just as in other regions of the central nervous system, fast excitatory synaptic transmission within the ACC is mediated by the excitatory amino acid glutamate (Sah and Nicoll 1991; Wei et al. 2001). Glutamate exerts its signaling role by acting on glutamate receptors, including N-methyl-D-aspartate (NMDA), 3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate, and metabotropic glutamate receptors (Dougherty et al. 1992). In the ACC, NMDA receptors are highly expressed, although their function remains unclear. Our early studies have shown that reverse microdialysis of AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) reduced basal and abolished CRD-induced ACC neuronal firing in normal rats. In contrast, microdialysis of NMDA receptor antagonist AP5 had no effect on ACC neuronal firing in normal rats. However, AP5 produced marked inhibition of ACC neuronal firing evoked by 50 mmHg CRD in VH rats (Wu et al. 2008). It appears that ACC nociceptive transmissions are mediated by glutamate AMPA receptors in the control rats. ACC responses to CRD are enhanced in VH rats. NMDA receptors mediate ACC synaptic responses after the induction of visceral hypersensitivity (Wu et al. 2008). Unlike other ionotropic receptors, NMDA receptors are 5–10 times more permeable to Ca^{2+} by way of NMDA receptors from the extracellular space into the postsynaptic cells, triggering a cascade of signaling molecules, including protein kinases, protein phosphatases, as well as enzymes that produce

diffusible retrograde messengers. The function of NMDA receptors as coincidence detectors and their permeability to Ca^{2+} makes these receptors the best candidates for a central mechanism in memory formation (Dougherty et al. 1992; Fan et al. 2009).

8.1.4 The ACC Plays a Critical Role in the Modulation of Behavioral Visceral Pain Responses in VH Rats

Pain contains both sensory and affective dimensions. Teasing apart the mechanisms that control the neural pathways mediating pain effect and sensation is a challenge. It is not clear whether activation of the ACC is only causally involved with the perception of pain-related unpleasantness or if it is also involved with the perceived intensity of pain during noxious visceral stimulation. Rodents do not have the fore-brain structures to generate the cognitive feelings of humans. The use of behavioral paradigms to assess spinal pain reflexes that do not include the assessment of cognitive perception in the conscious rat may help to identify the regulatory role of the ACC in visceral pain sensation. Based on brainstem reflexes, which have been described as “pseudoaffective” responses (Ness and Gebhart 1990), the nociceptive response (visceromotor response (VMR) to CRD) was recorded. Both control and VH rats showed pressure-dependent increases in the VMR to CRD. A significant VMR to the lowest distention pressure tested in VH rats and an absence of response to the lowest distention pressure in normal rats suggest a reduced pressure threshold (ie, allodynia) in VH rats (Fig. 8.2). These results provide evidence of enhanced visceral pain responses (ie, hyperalgesia) in VH rats (Fan et al. 2009; Cao et al. 2008). In this model, no significant mucosal inflammation in the colon 7 days after the initiation of visceral hypersensitivity were observed. The hypersensitivity to colonic distention, however, can be observed even up to 7 weeks following the initiation of colonic anaphylaxis and appears to be independent of mucosal inflammation (Fan et al. 2009). Hence, this may be a useful model to study post-inflammatory conditions of visceral hyperalgesia such as post-infection IBS, which occurs in up to 20% of patients following an acute bout of gastrointestinal infection.

Electrical stimulation of the rostral ACC in conscious rats enhances the VMR to CRD in a frequency-dependent manner. Furthermore, bilateral ACC lesion does not change the VMR in normal rats but markedly inhibits the VMR to CRD in VH rats. The reduction in the VMR after ablation of the rostral ACC suggests that neural networks in this region mediate allodynia and hyperalgesia in viscerally hypersensitive state (Gao et al. 2006). Further study showed that injection of low-dose glutamate into the ACC has no effect on the pain response in normal rats; however, in VH rats, it has a potent effect on the VMR to CRD, suggesting sensitization of glutamate receptors in ACC neurons in viscerally hypersensitive states (Cao et al. 2008). To determine the role of glutamatergic transmission in the modulation of visceral pain, investigators have shown that microinjection of the NMDA receptor antagonist AP5 into the ACC suppresses the CRD-induced increase in the VMR in VH rats

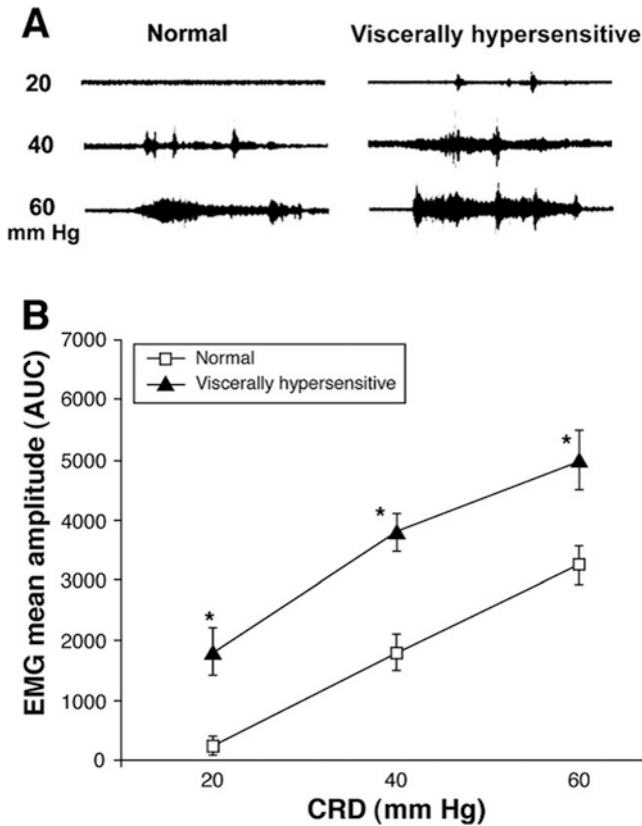


Fig. 8.2 VMR to graded distention pressures in normal control and viscerally hypersensitive rats. Under basal conditions (CRD, 0 mmHg), there was no significant difference between normal control rats and viscerally hypersensitive rats. (a) A representative abdominal muscle electromyogram of the VMR to graded-pressure CRD recorded from the external oblique pelvic muscle in normal and viscerally hypersensitive rats. (b) Mean amplitude of the abdominal muscle contraction expressed as AUC after baseline subtraction was presented. Data were collected from 7 sham-treated control rats and 8 viscerally hypersensitive rats. Analysis of variance showed a significant effect for distention level, as well as a significant interaction between distention level and group ($*P < 0.05$). Stimulus-response functions were shifted to the left in viscerally hypersensitive rats, indicating group differences in the VMR response. Values are presented as means \pm SE (Adapted from Cao et al., *Gastroenterology* 134:535–543, 2008. With permission.)

(Fan et al. 2009). It appears that visceral hyperalgesia in this rat model involves endogenous activation of descending facilitator pathways mediated by NMDA receptors in the rostral ACC (Gebhart 2004).

What makes glutamatergic synapses unique is that they can sustain synaptic plastic changes that may persist for hours to days (Dudek and Bear 1992). Further studies have to be conducted to explore the cellular and molecular mechanisms underlying these dynamic changes in ACC neurons in the viscerally hypersensitive state.

8.1.5 Up-Regulation and Phosphorylation of CaMKII Post-Synaptic Binding to NR2B Receptors Contributes Visceral Pain

NMDA receptors contain heteromeric combinations of the NR1 subunit plus one or more of the subunits NR2A–2D. Although NR1 is distributed widely in the brain, NR2 subunits show regional specificities. In the ACC, the NMDA receptor containing NR2A or NR2B subunits contributes to most NMDA-receptor currents. In human beings and rodents, the subunits NR2A and NR2B are dominant in forebrain structure (Monyer et al. 1994). In the ACC, the NMDA receptor containing NR2A or NR2B subunits contribute to most NMDA-receptor currents (Zhao et al. 2005). Mice that genetically overexpress the NR2B-receptor subtype in the forebrain show enhanced responsiveness to painful stimuli (Cao et al. 2008) and superior learning ability and memory of different behavioral tasks (Tang et al. 1999).

Considering the distinct roles that NMDA receptors may serve, identification of the receptor subtype in the ACC that mediates visceral hypersensitivity will promote our understanding of the molecular mechanisms underlying nociceptive processes in the VH state. The up-regulation of NR2B-receptor protein was verified by Western blot analysis in VH rats (Fan et al. 2009) (Fig. 8.3). On the other hand, no significant increases in NR1 and NR2A protein expression were observed (Fan et al. 2009).

Electrophysiological studies showed that reverse microdialysis of NVP-AAM077, a specific NR2A-subunit antagonist, had no effect on basal and CRD-induced ACC neuronal firing in VH and control groups. In VH rats, Ro25-6981, a specific NR2B-subunit antagonist, inhibited ACC neuronal firing, evoked by 30 and 50 mm Hg CRD, by 98% and 52% respectively. Behavioral studies showed that neither NVP-AAM077 nor Ro25-6981 changed the VMR to graded-pressure CRD in normal rats (Fan et al. 2009). On the other hand, in VH rats, NVP-AAM077 had no effect on the VMR, whereas Ro25-6981 dose-dependently decreased the VMR to CRD suggesting that NMDA NR2B-receptor activities in the ACC are responsible for allodynia and hyperalgesia in VH rats. To down-regulate NR2B-receptor gene expression, an NR2B-specific small interfering RNA (siRNA) and a plasmid (pEGFP-N1) that expressed the green fluorescent protein were administered into ACC neurons by electroporation. NR2B siRNA-treated VH rats showed a significant reduction in the VMR, compared with controls indicating that the NR2B subunit of NMDA receptor activation of sensitized ACC neurons plays a causative role for the long lasting visceral pain responses, which are independent of inflammation in the colon in the functional viscerally hypersensitive rats (Fan et al. 2009; Cao et al. 2008).

The NMDA receptors may play important roles in plasticity and memory via multiple downstream signaling pathways including calcium/calmodulin-dependent protein kinase II (CaMKII) (Lisman et al. 2002). CaMKII is enriched at the post-synaptic density (PSD). Localization of CaMKII at the PSD has been proposed to play a critical role in long-term potentiation (LTP) (Lisman et al. 2002). CaMKII is

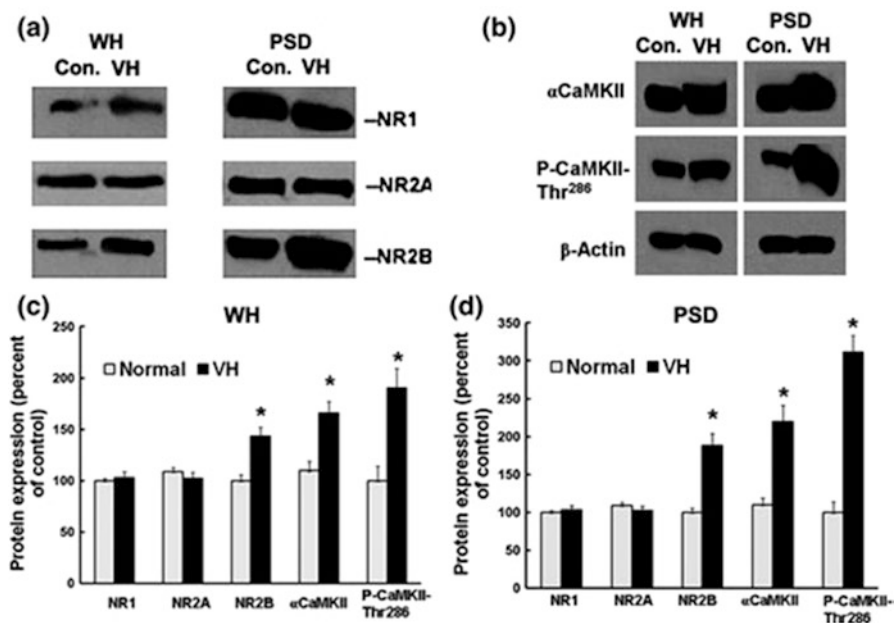


Fig. 8.3 Expression of subtypes of NMDA receptor, aCaMKII and PaCaMKII-Thr286 in the pACC. (a) Representative immunoblots from pACC showed the expression of NMDA receptor subunits NR1, NR2A and NR2B in WH and PSD extracts in the normal and VH rats. Western blot showed that the expression level of NR2B, but not NR1 or NR2A was increased significantly in the pACC of VH rats 10 days after the induction of visceral hypersensitivity. (b) Representative Western blots showed enhanced expression of aCaMKII and P- α -CaMKII-Thr286 in WH and PSD extract in VH rats compared with control. (c, d) Relative intensities of NR2A, NR2B, α CaMKII and P-aCaMKII-Thr286 protein were measured by densitometry analysis. Quantification of protein expression in the pACC of VH rats was expressed as the percentage of controls. Each column represented the means \pm SEM. Statistical significance was determined by Student's t-test between normal and VH rats for each molecule. * $p < 0.05$, $n = 6$ for each group (Adapted from Li et al. *J Neurochemistry*, 1111/j.1471-4159. 2012. With permission.)

autophosphorylated at Thr286, and may function as a biochemical 'memory' process. An elegant study showed that NR2B binding targets activated CaMKII in the PSD and maintained persistent activity of this kinase in subcellular compartments (Bayer et al. 2001). A series of studies using a biochemical fractionation approach showed over-expression of NR2B and CaMKI in the ACC, and post-synaptic accumulation of NR2B and CaMKII in the ACC synapses. Further investigation showed the increases in phosphorylated CaMKII (Thr286CaMKII) protein level in the post-synaptic density fraction (PSD) (Triton X-100 insoluble) and extrasynaptic (Triton X-100 soluble) fractions (Li et al. 2012). Western blotting following co-immunoprecipitation showed that phosphorylated-CaMKII-Thr286 bound to NR2B in the PSD, which was increased to 267% of control in VH rats (Fig. 8.4). Administration of CaMKII antagonist Antennapedia-CaMKIINtide suppressed visceromotor response in VH rats, and in parallel, restored the level of NR2B to control

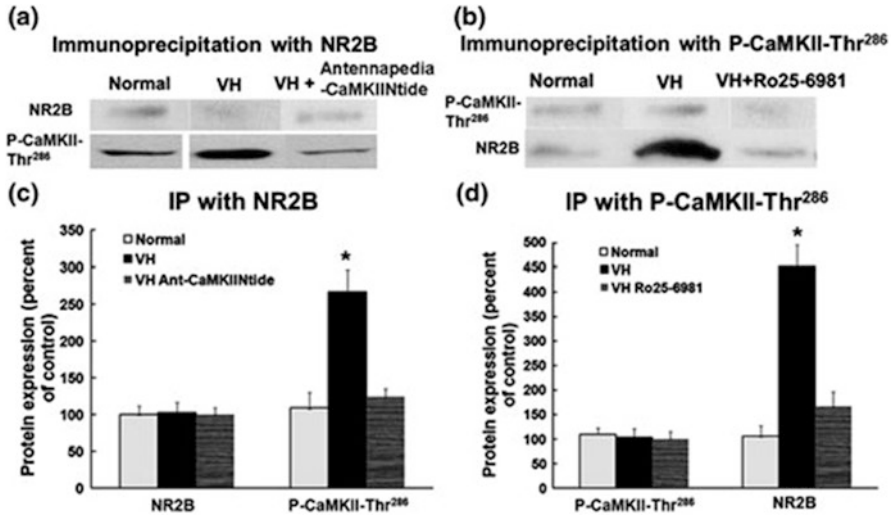


Fig. 8.4 Enhanced post-synaptic association between active α CaMKII and NR2B in the VH rats. (a) PSD fractions of control, VH and VH rats treated with Antennapedia-CaMKIIntide were isolated. Samples were immunoprecipitated with NR2B antibody before immunoblotting with P-CaMKII-Thr286 antibody. (b) PSD fractions of control, VH and VH rats treated with Ro25-6981 were isolated. Samples were immunoprecipitated with P-CaMKII-Thr286 before immunoblotting with NR2B antibody. (c, d) Summary graphs showed increased interaction between P-CaMKII-Thr286 and NR2B in the VH rats. Each column represented the means \pm SEM. Statistical significance was determined by one-way ANOVA followed Bonferroni post-tests. * $p < 0.05$, $n = 5$ for each group (Adapted from Li et al. *J Neurochemistry*, 1111/j.1471-4159. 2012. With permission.)

levels and reduced the NR2B-P-CaMKII-Thr286 protein complex in PSD. To down-regulate the gene expression of CaMKII, RNA-specific interfering RNA (siRNA) was administrated into the pACC neurons of VH rats by in vivo electroporation. This treatment completely abolished the increases in CRD-induced VMR (Li et al. 2012). All these data imply the close relationship between CaMKII and NR2B in ACC in chronic pain, namely, CaMKII might act as an amplifier of detrimental cellular calcium signal regulated by NMDA receptors when becoming autophosphorylated and targeting to NR2B; conversely, autophosphorylated CaMKII could modulate post-synaptic NR2B subtype of NMDA receptor localization. Data from these series of experiments supports the hypotheses that CaMKII is a critical signaling molecule in the ACC glutamatergic synaptic transmission, phosphorylation of CaMKII at Thr286, which binds to NR2B subunit at post-synaptic site, modulates visceral pain in viscerally hypersensitive state.

8.1.6 Perigenual ACC (pACC) Are Necessary for the “Aversiveness” of Visceral Nociceptor Stimulation

Pain contains both sensory and affective dimensions. Except for human experiments where self report is possible, teasing apart the mechanisms that control the neural pathways mediating pain affect and sensation in an overall animal nociceptive behavioral response is a challenge. It is well documented that the ACC is involved in pain processing and encoding of negative affect in humans, which results in pain-related unpleasantness (Tolle et al. 1999). Research suggests that ACC neuronal activity in rodents is related to stimulus–reward learning (Bussey et al. 1997). Johansen, Fields, and Manning (Johansen et al. 2001), and Johansen and Fields (Johansen and Fields 2004) introduced a formalin-induced conditioned place avoidance (F-CPA) model to distinguish somatic pain emotion from pain sensation in rats. Using a rodent visceral pain assay that combines the colorectal distension (CRD)-induced visceromotor response (VMR) with the conditioning place avoidance (CPA) researchers in the field of visceral pain, Yan et al. measured a learned behavior that directly reflects the affective component of visceral pain (Yan et al. 2012). When CRD was paired with a distinct environment context, the rats spent significantly less time in this compartment on the post-conditioning test days as compared with the pre-conditioning day. Effects lasted for 14 days. Bilateral ACC lesion significantly reduced CPA scores without reducing acute visceral pain behaviors (CRD-induced VMR). Bilateral administration of non-NMDA receptor antagonist CNQX or NMDA receptor antagonist AP5 into the pACC decreased completely abolished the CPA in the day 14 after conditioning. These data suggested that pACC activation is critical for the memory processing involved in long-term negative affective state and prediction of aversive stimuli by contextual cue.

8.2 ACC Synaptic Plasticity Mediates Learning and Long-Lasting Functional Visceral Pain Memory

Using the visceral hypersensitivity rat model, a series of behavioral studies suggested that the facilitation of visceral pain responses following a brief noxious stimulus (colonic anaphylaxis in our model), the hypersensitivity to colorectal distension (CRD) can be observed up to 7 weeks after the initiation of colonic anaphylaxis. The chronic visceral pain was independent of mucosal inflammation, suggesting mediation by a mechanism for the learning and triggering of memory, where information needs to be stored and retrieved. However, the mechanisms underlying how visceral nociceptive input is encoded within the ACC have not been explored. The synaptic substrates in the ACC neuronal circuitry responsible for storing visceral nociceptive information for prolonged periods of time (e.g. by use-dependent change in synaptic strength) have not been identified. A key insight in neuroscience over the past three decades is that synaptic connections between neurons are in a

near-continual state of change (Bliss and Collingridge 1993). The responses of neocortical neurons can be persistently modified by alterations in sensory experience. Such modifications reflect changes in synaptic transmission that shape cortical circuits and store information (Martin et al. 2000). At the cellular level, activity-dependent plasticity in synaptic strength, such as long-term potentiation (LTP) and long-term depression (LTD), may serve as key synaptic mechanisms reflecting cortical plasticity (Bliss and Collingridge 1993; Chapman et al. 1998).

8.2.1 Visceral Hypersensitivity Is Associated with Alterations of the Properties of Synaptic Plasticity in the ACC

The medial thalamus (MT) serves as a major relay in the medial pain system and in the conveyance of nociceptive information to the ACC (Vogt et al. 2003; Shyu and Vogt 2009). There is wealth of evidences to support the view that the synapses mechanisms contribute to learning, and memory storage for long lasting functional visceral pain. LFP is a low frequency (40–130 Hz) component of the electrophysiological signal. It reflects the superposition of synchronized dendritic currents, averaged over a large variety of interneurons and intracortical activity. FP recording is one way to study artificially induced synaptic plasticity (Martin et al. 2000; Shyu and Vogt 2009). The ACC FPs elicited by electrical stimulation of the MT were used as a quantitative measure of synaptic strength. I/O curves generated by a gradual increase of the stimulus intensity (50–1000 μA) showed significant increases in LFP in the sensitized rat suggesting enhancement of basal synaptic transmission in the thalamo-ACC synapses after induction of visceral hypersensitivity; this is mediated by both NMDA and AMPA receptor activity (Wang et al. 2013).

Electrophysiological recordings from animals and humans have revealed that ACC neurons are likely to fire action potentials at 4–7 Hz (theta) during various behavioral tests (Nishida et al. 2004). Electrorheological study in the ACC area have shown that theta burst stimulation (TBS) reliably induces LTP-like plasticity in the MT-ACC pathway in normal rats. However, in the VH state, the expression of LTP-like plasticity in MT-ACC synapses was smaller or occluded. An additional study by using low-intensity stimulus, which evoked response in VH rats that is comparable to the response induced by 400 μA (induced 50% of maximum amplitude of the LFP) in control rats. We found that low intensity stimulus in VH rats also failed to elicit increases in LFP amplitude following TBS conditioning suggesting that the induction of LTP-like plasticity in the MT-ACC synapses was blocked in the VH states (Wang et al. 2013). It appears that, in the VH state, transduction signals in the MT-ACC synapses are not available for subsequent electrical recruitment, suggesting that the synaptic strengthening occurring in the VH state engages signal transduction pathways that are in common with those activated by electrical stimulation.

8.2.2 *Visceral Hypersensitivity vs ACC Synaptic Plasticity: Chicken or Egg?*

To further characterize whether LTP-like synaptic plasticity in the MT-ACC synapses contributes to visceral pain in VH state, we showed that repeated artificial induction of LTP in the MT-ACC synapse by chronic repeated theta-patterned tetanization in the MT in normal rat (Wang et al. 2015) facilitated behavioral visceral pain, which mimic visceral allodynia and hyperalgesia in the VH model (Fan et al. 2009; Cao et al. 2008). These observations lend support to the theory that the enhanced long-lasting transmission at the MT-ACC synapses *causally contributes* to visceral pain. It appears that chronic visceral pain, ACC sensitization and long-lasting enhanced synaptic transmission in the VH state are expressed by the same core mechanisms as TBS-induced canonical LTP in rats (Wang et al. 2013). ACC synaptic strengthening may engage signal transduction pathways that are in common with those activated by TBS, and serves as an attractive cellular model of functional visceral pain. It appears that induction of chronic visceral pain produces a change in the ability to induce subsequent synaptic plasticity at the ACC neural circuitry, we hypothesize that the mechanisms of ACC synaptic metaplasticity not only are involved in the processes of modifying the visceral pain sensitivity (Fan et al. 2009; Cao et al. 2008) and the aversive responses to pain (Yan et al. 2012), but also further affect the processing of learning and memory in chronic pain state (Wang et al. 2013, 2015).

8.3 Visceral Pain and Cognitive Deficits

Pain is a perception rather than as a purely sensory modality, and it is worth mentioning that for pain to be consciously experienced, cognitive processing is required. The conceptualization of pain in humans recognizes the components involved in the encoding and perception of stimulus parameters (e.g., stimulus localization, intensity, and quality), and the affective salience or unpleasantness of the noxious stimulus. Chronic pain (defined as pain persisting for 3–6 months or longer) generally exceeded the duration of the precipitating noxious stimulus or injury, and is associated with the development of affective disorders but the underlying mechanisms are not fully understood. Ample clinical evidence has shown that most of the patients with IBS who seek treatment have psychiatric comorbidity, notably depression and anxiety disorders (Larsson et al. 2012; Longstreth et al. 2006). Symptoms of major depressive disorder (MDD) occur in up to 90% of patients with IBS (Friedrich et al. 2010), clinical evidence has also shown that chronic pain patients usually suffer from memory deficiency, so it is surprising that there has been no experimental animal model to study visceral pain related emotional disorder and cognitive deficits, and little is known about the underlying mechanisms.

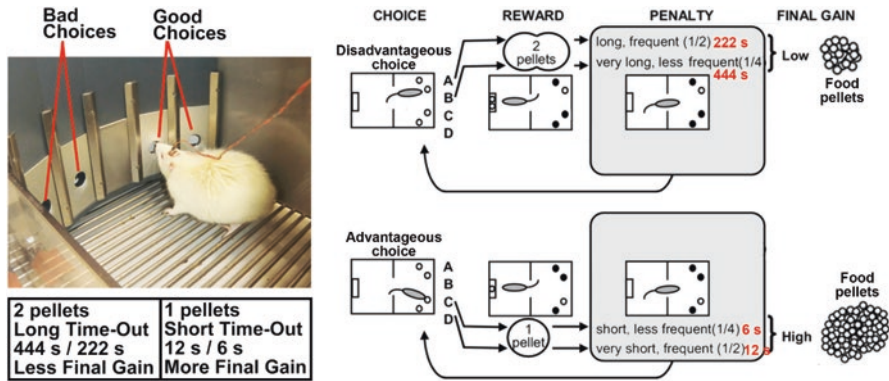


Fig. 8.5 Rat gambling task procedure. During the gambling task, rats can nose-poke either one of the four holes, A, B, C or D to obtain reward. The RGT is used to test the decision-making by choosing between options associated with different amounts of reward in terms of food pellets at different likelihood/probability of penalties, which will be time-outs in this case. With the choice A or B, the rat will have a reward of two food pellets each time but it will either experience a long and frequent or a very long and less frequent timeout, the final gain will be less compare to the choice C or D. Although the rat will only have one food pellet in reward by choosing C or D, it will only experience either a short and less frequent time-out or a very short and frequent time-out. This pattern is advantageous and it will have more food rewarded at the end (Adapted from Cao et al., *Experimental Neurology* 136 (2016) 74–85. With permission)

Cognition refers to a set of mental processes, including attention, memory, evaluation, decision-making, etc. Making a decision under uncertain conditions is a basic cognitive process for adaption relying on the integration of several executive functions. Impaired decision making has been demonstrated to represent a key symptom in many mental disorders. In humans, decision-making has been accurately modeled using the Iowa gambling task (IGT) (Bechara et al. 2000, 1997). In behavioral tasks for animals, several psychiatric symptoms addressing higher-order cognitive dysfunctions have been reproduced (Nestler 2006). Rodents are individuals which can exhibit human-like cognitive characteristics, such as the ability to learn and reason with causal knowledge (Blaisdell et al. 2006). Rodent models of decision-making, such as rat gambling task (RGT), are particularly valuable as experimental conditions can be controlled (Zeeb and Winstanley 2011; Xu et al. 2015; Mu et al. 2015; Cao et al. 2016).

8.3.1 *Visceral Hypersensitivity Affects Decision-Making Ability in Rats*

The RGT has been developed to test the decision-making capacities in rats via a conflict between immediate and long-term gratification (food reward). Operant chambers (28 × 30 × 34 cm) were used for RGT (Fig. 8.5). During the training

stage, rat gradually learned the association between the nose-poke action and the release of food pellet in the food tray. The training phase usually lasted 7–10 days. The 60-min test was performed the following day. Rats were allowed to freely make choices among the four apertures (A–D) as they did in the training phase, however, different choices were associated with different outcomes. Although the immediate reward of choice A and B was two times of C and D, in the long run, the theoretical maximum benefit of C and D will be five times higher than A and B. The rats selected all 4 different options equally during the first 10 min. Over time, good decision makers progressively developed a preference for the advantageous options. The proportion of advantageous choices (%) = numbers of nose-poke (C + D) / nose-poke (A + B + C + D) * 100% were used to identify decision-making behavior of rats. Following criterion was used to distinguish the good (>70% preference), delayed-good (>70% preference in the last 20 min), undecided (30–70%), and poor (<30%) decision-makers during the last 20 min (Fig. 8.5). At the end of the testing, good decision-makers earned significantly more food pellets across the session. Studies of brain-lesion or psychiatric patients have discovered the specific prefrontal cortex (PFC) areas mediating decision-making (Bechara et al. 2000; Rushworth et al. 2007). Animal studies have shown that decision-making performances in the RGT depend on the integrated function of several sub-regions of the PFC, especially the prelimbic, cingulate and orbitofrontal cortices, and amygdala (Zeeb and Winstanley 2011). Recently, using chronic visceral pain rat model the difference in the proportions of the subgroups between the control and (VH) groups was reported (Flood et al. 1987) (Fig. 8.6). The significant decreases of the proportion of good decision-makers from 71% in the control to 46% associated marked increases in indecisive decision-makers discovered in the chronic visceral pain rats (Flood et al. 1987). These data provide the first evidence that chronic visceral pain led to decision-making deficits in rats.

8.4 ACC Neuronal Spike Field Phase Locking and Synchrony Cross Areas Associated Involved in the Processing of Chronic Visceral Pain

Neuronal oscillations are likely to be a fundamental mechanism for modulating, filtering, and redirecting information in the nervous system. In the last few years, large scale neural oscillations have been acknowledged to play a primary role in fundamental cognitive functions. Ample evidence suggests that neurons transmit information not only by changing their firing rates but also timing of the spikes corresponding to the ongoing neuronal oscillations (Varela et al. 2001).

Furthermore, the induction of synaptic plasticity is favored by coordinated action potential timing across neuronal networks (Markram et al. 1997), giving rise to oscillations of different frequencies in the local field potential (LFPs). These field potential oscillations have been shown to modulate local spike timing (Jacobs et al. 2007).

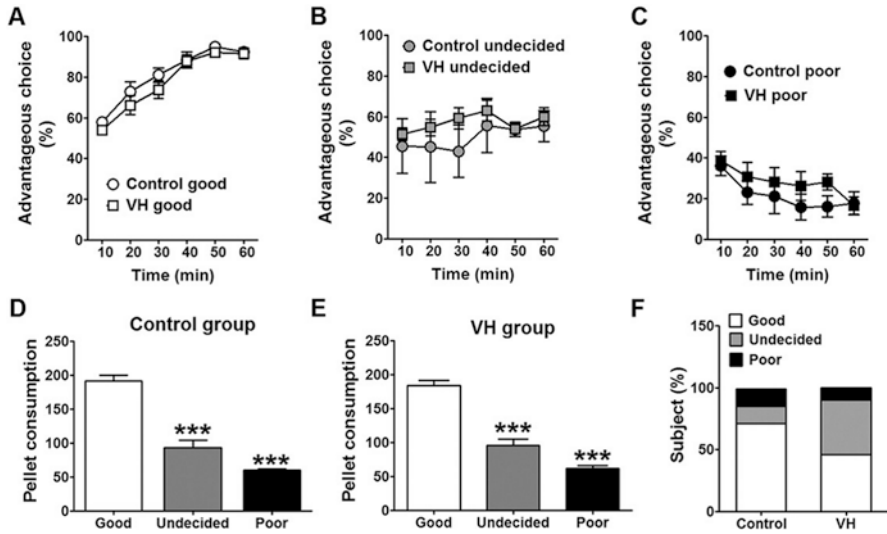


Fig. 8.6 Changes in decision-making behavior induced by visceral hypersensitivity using a rat gambling task. (a–c) Mean time-course of advantageous choices of good (a), undecided (b) and poor (c) decision-makers during the RGT. (d, e) Total pellet consumption during the 60 min RGT testing for good (white), undecided and poor (black) decision-makers of control rats and VH rats. *** $p < 0.001$ vs. good decision-makers. (f) Proportions of good (white bar), undecided and poor (black bar) behavior was represented for control group and VH group. Advantageous choices (%) = numbers of nose-poke for choices (c + d)/numbers of nose-poke for choices (a + b + c + d) $\times 100\%$. $n = 28$ for control group, $n = 39$ for VH group (Adapted from Cao et al., *Experimental Neurology* 136 (2016) 74–85. With permission.)

8.4.1 Tight Coordination of Spike Timing with the Local Theta Oscillation Is a Key Index for Predicting Successful Cognitive Function

Rutishauser et al. have shown that memory formation in humans is predicted by close coordination of spikes phase-locking with the theta band local field potentials (Rutishauser et al. 2010). Within individual brain areas, oscillations can synchronize neurons, creating coherent cell assemblies (Harris et al. 2003) and appropriate plasticity depending on the precise timing of pre- and post-synaptic activity (Markram et al. 1997; Fig. 8.7). Evidence points to cortical oscillations as a mechanism for mediating interactions among functionally specialized neurons in distributed brain circuits. A brain function that may use such interactions is declarative memory (Rutishauser et al. 2010)—that is, memory that can be consciously recalled, such as episodes and facts. A growing body of evidence clarified by us and others suggests that cortical oscillation at theta band, in particular, the synchrony between spike timing and theta oscillation facilitates

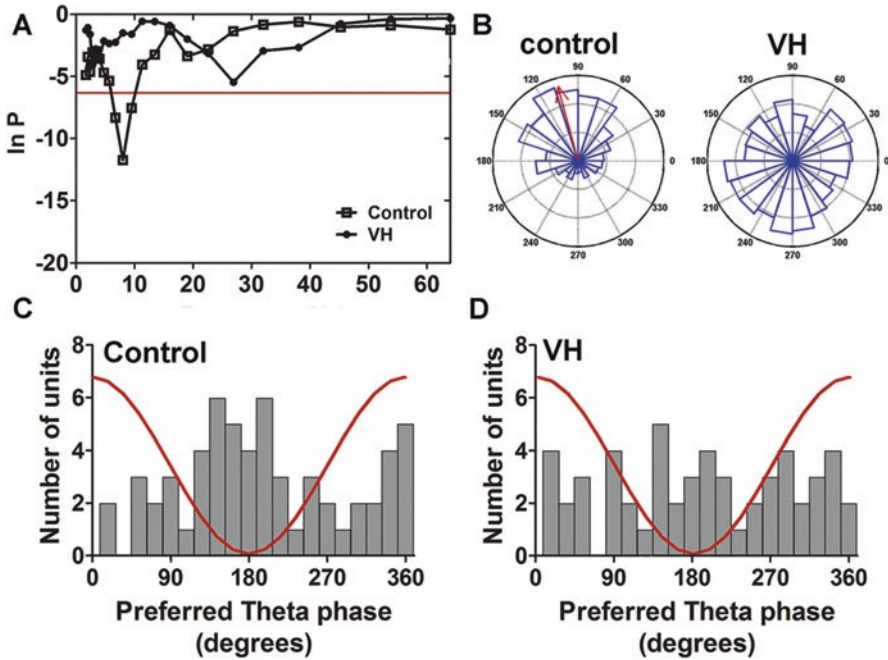


Fig. 8.7 VH disrupted the spikes phase-locking to theta band oscillation in the ACC. (a) Test of significance of phase-locking as a function of frequency (1–64 Hz). The threshold (red line) for significant phase-locking was set to $p = 0.0023$ ($0.05/22$, Bonferroni corrected). The shown phase-locked neuron in the control rat exhibited maximal phase-locking at 8.0 Hz while the other un-phase-locked neuron in the VH rat showed no significant phase-locking in any frequencies. (b) The polar-histogram of the spike-field phase distribution of the phase-locked neuron from the control rat and un-phase-locked neuron from VH rat, which are shown in (a). The mean phase showed by red arrows also indicated this neuron preferred firing at 105° of the theta oscillation, while the other un-phase-locked neuron in VH rat showed no significant phase-locking in theta range. (c) Histogram of the preferred phase of all phase locked neurons ($n = 59$ of 114) recorded in the 6 control rats. The figure shows most neurons preferred to fire during the descending phase and at the trough of the oscillations. The red line is a schematic of one theta cycle. (d) The phase locked neuron ($n = 54$ of 217) recorded in 6 VH rats, however, fired action potential at random angles of the theta cycle in the oscillations suggesting disrupted phase-locking relationship between action and field potentials in rats following VH (Adapted from Cao et al., *Experimental Neurology* 136 (2016) 74–85. With permission.)

neuronal communications, modifies synaptic weights between anatomically distant, but functionally associated brain regions, and related to even behavioral outputs (Xu et al. 2015; Mu et al. 2015; Cao et al. 2016; Cardoso-Cruz et al. 2013).

8.4.2 *Interruption of Amygdala-ACC Integrative Coordination Contribute Causally to Cognitive Dysfunctions in Chronic Pain States*

It has been shown that the basolateral amygdala (BLA) and the ACC form an interconnected neural circuit that may mediate certain types of decision-making processes (Floresco and Ghods-Sharifi 2007). Recently, it was reported that pain related hyperactivity of basolateral amygdala neurons mediates decision making deficits through the amygdala-prefrontal cortex circuit, suggesting that cognitive impairment is caused by amygdala-driven prefrontal cortical deactivation. The reciprocal connections between the BLA and medial PFC including the ACC have been clearly exhibited previously (Bacon et al. 1996). We have performed multiple-channel electrophysiological recordings and adopt standard multi-channel data analyses, such as cross-correlations and spectral analyses for local field potential (LFP) and spike recordings, to characterize the spike-field coherence (SFC), and phase locking of individual neurons to the theta oscillation within each regions, and between ACC and basolateral amygdala (BLA). Our published data showed that phase-locking and synchronization in ACC and between ACC and amygdala play a major role in modulation of cognition function in various preclinical animal models (Wang et al. 2015; Xu et al. 2015; Mu et al. 2015; Cao et al. 2016).

In viscerally hypersensitive rats, recordings of field potential showed facilitation of basal synaptic transmission in the BLA-ACC pathway, suggesting up-regulation of long lasting synaptic transmission in the ACC neural circuitry following induction of visceral hypersensitivity (Zeeb and Winstanley 2011). Previous study showed that BLA efferent exerted a predominantly inhibitory effect (Perez-Jaranay and Vives 1991). In line with this observation, recent study showed that there was a reliable induction of LTP at the BLA-ACC synapses in normal rats. However, the LTP in the BLA-ACC synapses was blocked in VH rats (Zeeb and Winstanley 2011). It appears that induction of visceral hypersensitivity produces a change in the ability to induce subsequent synaptic plasticity at the BLA-ACC pathway. Further, power spectral density analysis showed an increase in accumulative power of the theta band of LFP in both the BLA and ACC in VH rats that was associated with a marked decrease of theta peak frequency (Flood et al. 1987). In fact, the increases in theta power and the shift of the dominant peak of theta to lower frequencies have been proposed as markers of cognitive decline in chronic pain (Cardoso-Cruz et al. 2013; Sarnthein et al. 2006). Cross-correlation analysis revealed visceral hypersensitivity led to suppressed synchronization of theta oscillation between the BLA and ACC (Fig. 8.8) suggesting that they loosely interact for dynamic information transfer, which may in turn disrupt neural network assemblies and affect synaptic plasticity. Finally, we observed suppressed locking of ACC spikes to the phase of the theta oscillations in the BLA in the VH rats. The SFC analysis is independent of the LFP power spectrum and the number of spikes, and is therefore immune to changes in these parameters. These findings are particularly intriguing in view of the recent findings that a tight coordination of spike timing with the local theta oscillation is a key index for predicting successful memory formation in humans (Rutishauser et al. 2010).

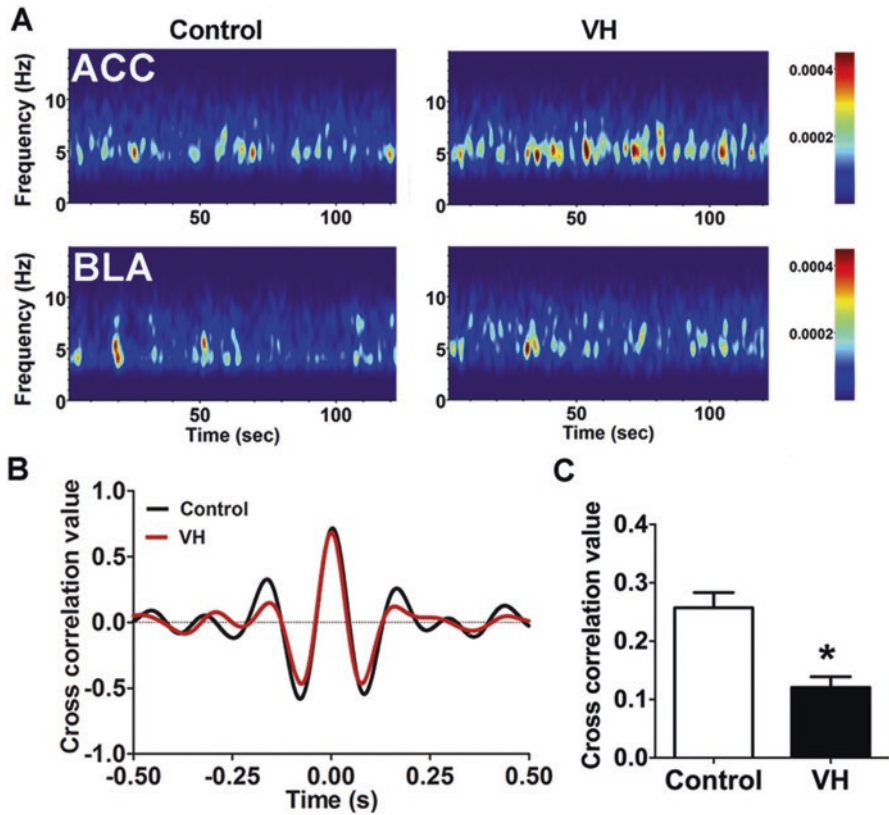


Fig. 8.8 Synchronization between theta oscillations in the basal lateral amygdala (BLA) and anterior cingulate cortex (ACC). (a) Typical colored power spectrograms (120 s duration) recorded from ACC (top) and BLA (bottom) in control rats (left) and VH rats (right). Note that theta power decreased in the ACC but increased in the BLA of VH rat compared with control rat. (b) The averaged cross-correlograms in control and VH rats at quiet waking state. (c) Statistic analysis revealed that the cross-correlation value (the second positive peak in b), which is corresponding to theta activity, decreased in VH rats compared to control rats, $*p < 0.05$

8.5 Vagus Nerve Stimulation Modulates Neuronal Spike Field Phase Locking and Synchrony Cross Areas Associated with Facilitation of Decision-Making in Rats

The viscerosensation is a faculty of perception that does not depend on any outward sense (Zagon 2001), which acts to influence the elicited behavioral response. Vagus Nerve Stimulation (VNS) is used clinically as a treatment for refractory epilepsy (Elger et al. 2000), and resistant depression (Nemeroff et al. 2006). VNS has also shown several beneficial effects for mood enhancement (Elger et al. 2000; Beekwilder and Beems 2010), and promoted cognitive functions in Alzheimer's patients (Sjögren et al. 2002). Clark et al. have shown in human patients VNS at intensity comparable to that effective in rodents facilitated retention of verbal learning performance (Clark et al. 1999). In rats VNS (0.4 mA) given immediately after training enhanced retention performance on an inhibitory-avoidance task (Clark et al. 1998). Using behavioral paradigm to evaluate visceral pain in conscious rats, we have demonstrated that subdiaphragmatic vagus nerve stimulation has visceral analgesic properties in rats (Chen et al. 2008), furthermore, visceral pain-related affective memory was enhanced by VNS (Zhang et al. 2013).

8.5.1 Vagal Nerve Stimulation Enhances Cognitive Performance and Facilitate Decision Making

Making a decision under complicated and uncertain conditions is a basic cognitive process for adaption relying on the integration of several executive functions. In humans, decision-making has been accurately modeled using the Iowa gambling task (IGT) in the laboratory (Bechara et al. 1999, 2000). Previous report showed that subjects with spinal cord injury (second to sixth cervical vertebra) did not show dysfunctions in decision making (North and O'Carroll 2001) suggesting changes in sympathetic activity are not critical to determining somatic tone, and affect decision making. A preliminary, but impressive experimental study by Martin et al. (Martin et al. 2004) has shown that VNS improved decision making in medical refractory epileptic patients. Together, these lines of evidence provide compelling rationale to hypothesize that activation of vagal afferent nerves may play an important role in the process of decision-making.

By employing a conscious rat model equipped with vagus nerve cuff electrode, we assessed ACC the role of chronic VNS on decision-making in rat gambling task (Fell and Axmacher 2011). The average food intake per body weight was not significantly different between the control (sham EVS) and EVS rat groups. Daily VNS, administered immediately following training sessions of RGT, caused an increase in 'good decision-maker' rats. The difference in the proportions of the three types of decision-making behavior (good, bad, undecided) between the two groups was

significant. The mean food reward obtained during the RGT by the VNS rats was significantly more than that of the controls (Fell and Axmacher 2011).

8.5.2 Vagal Nerve Stimulation Regulates LFP and Spike Phases, Enhances Spike-Phase Coherence Between Key Brain Areas Involved in Cognitive Performance

Simultaneous multichannel-recordings offer an ideal setup to test the hypothesis that VNS may induce alterations of in both spike-field-coherence and synchronization of theta oscillations across brain areas. Indeed, it has been reported that VNS augmented theta activity in BLA and ACC (Fell and Axmacher 2011). Spike-field coherence (SFC) was used to quantify the alteration in the spike timing-LFP relationship within the BLA and ACC before and after VNS. The SFC value is expressed in percentage and varies as a function of frequency. We found a significant difference in the average SFC after VNS in the theta range. No significant changes were observed in the other three frequency bands. To further examine the functional connectivity between the ACC and BLA, we compared the LFP during 30 s spontaneous periods from before and after VNS in rats. Cross-correlation and time-varying power spectral analysis of the theta oscillations revealed a pattern of dispersion of theta band activity during the basal period, and increases in correlation values immediately following VNS. Moreover, the increased LFP-synchronization between the BLA and the ACC was also associated with greater locking of ACC spikes to the phase of the theta oscillations in the BLA (Fig. 8.9). It is clear that communication between brain areas involves phase synchronization of oscillations (Fries 2005; Palva et al. 2005). The phase of the oscillation regulates exactly when gatherings of neurons spike, thus two brain areas with increased phase synchrony will have improved synaptic interaction and information exchanges (Fell and Axmacher 2011). It appears that the sequential transfer of information via corticopetal BLA/ACC connections may guide response variety when assessing the value of an anticipated outcome (decision making) relative to the costs of a particular action.

These electrophysiological evidences unveil several important roles for VNS in regulating LFP and spike phases, as well as enhancing spike-phase coherence between key brain areas involved in cognitive performance. These data may serve to provide fundamental notions regarding neurophysiological biomarkers for therapeutic VNS in cognitive impairment. Further studies are wanted to clarify what physiological stimuli activate the vagal afferents that modulate decision-making. For instance, Cholecystokinin-octapeptide (CCK-8), which is a gastrointestinal hormone released during feeding (Li et al. 2000; Li and Owyang 1996), acts on vagal afferent fibers. Our previous electrophysiological studies in rats have demonstrated that CCK stimulates vagal afferent fibers (Li et al. 2004, 1999) to modulate various gastrointestinal functions. Flood et al., have shown that administration of CCK-8 acts on vagal afferents to enhance memory retention in the mice after aversive

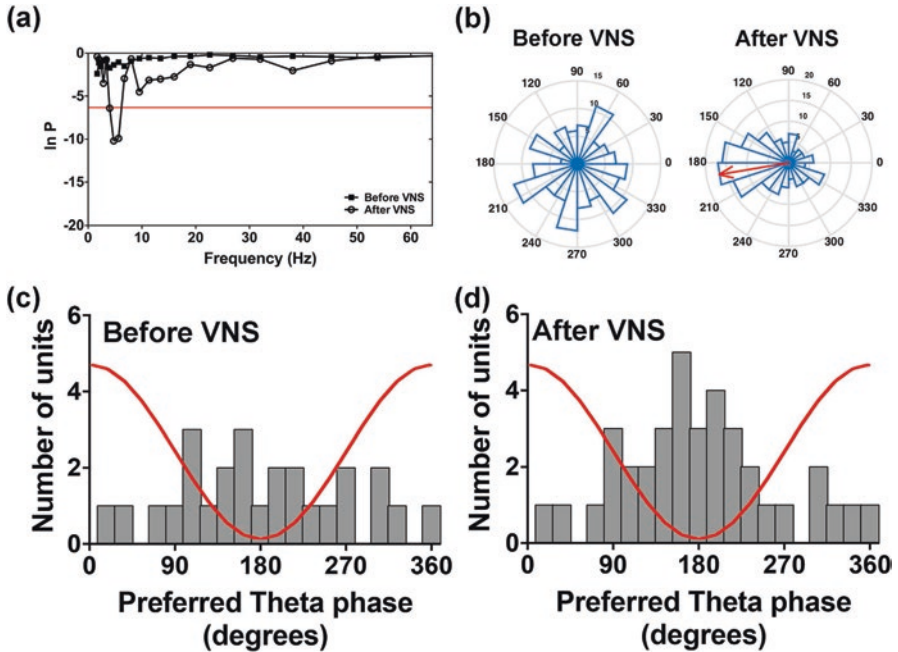


Fig. 8.9 Phase-locking of the spikes of the ACC neurons to the theta oscillations in the BLA. (a) Test of significance of phase-locking. The neuron which was un-phase-locked before VNS exhibited maximal phase locking at 5.65 Hz after VNS. (b) The polar histograms of the spike-field phase distribution of the same neuron shown in (a). The un-phase-locked neuron fired randomly before VNS (left panel) and became phase-locked to the theta cycle at 189° after VNS (right panel, vector length $R = 0.53$). (c, d) Histogram of the distribution of the preferred phases of all phase-locked neurons before and after VNS, $n = 26$ in (c) and $n = 37$ in (d). More phase locked neurons preferred to fire close to the trough of the oscillation (Adapted from Cao et al., Scientific Report)

training (Flood et al. 1987). Further studies are needed to determine if CCK enables or modulates cognitive function, such as decision-making, by acting on vagal afferent fibers.

8.5.3 Final Remark

The pain is likely to be reflected in a matrix of neuronal structures rather than in a fixed pain center. A “neuromatrix” incorporating the ACC, prefrontal cortex and the amygdala may be involved in the processing of pain without any single region unto itself being necessary and sufficient for the pain experience. It is conceivable that the course of the processes of neuron specialization during induction of visceral hypersensitivity are associated with changes in synaptic efficiency in the same cells during the induction of canonical LTP. This suggests the possibility that the same

synaptic mechanisms are involved in the processes of modifying ACC cells, reducing the pain threshold, amplifying affective responses to pain, and processing learning and memory in patients with IBS.

The visceral pain experience may be better explained as a biopsychosocial model of pain, although most clinical specialists continue to treat visceral pain as just a symptom and not as a distinct neurological entity. In the chronic visceral pain, state hyperactivity of amygdala effectively blocks the expression of canonical long term potentiation at BLA-ACC synapses. The impaired ACC LTP and the dysfunction of ACC intra-, and between areas spike field coherence play an important role in emotional disorders, and cognitive deficits, such as decision making, in the visceral hypersensitive state.

More recent findings reveal that vagus nerve stimulation induces between-area phase synchronization in theta frequencies and elevated phase locking of neuronal spike firings to theta oscillations across regions, and are perhaps candidates for explaining the neural mechanisms underlying VNS-facilitation of decision-making. The data will serve as a basis for fundamental notions regarding neurophysiological biomarkers for the development of novel therapeutics of cognitive deficits in the chronic visceral pain.

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