Spectral and correlation analyses of the verapamil-induced conversion of ventricular fibrillation to tachycardia in isolated rat hearts.

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Short Title: verapamil-induced conversion of VF to VT

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Abstract

Ventricular tachycardia (VT) is considered to be the most common precursor of ventricular fibrillation (VF). However, the mechanisms underlying the transition from VT to VF remain unclear. Here we investigated whether and how perfusion of the heart with verapamil, a blocker of L-type calcium channels, changed the macro-dynamics of the heart between VT and VF. The experiments were performed using Langendorff perfused isolated rat hearts, in which left ventricular pressure (LVP) and left ventricular cardiomyogram (LVCMG) were measured. Sustained VT or VF was induced by burst pacing of the left ventricular muscles. During sustained VF, verapamil perfusion resulted in the conversion of VF to VT. A cross-correlation analysis between LVCMG and LVP revealed that the correlation coefficient was small during VF, but became larger during VT. This study demonstrated that verapamil treatment converted sustained VF to VT, and characterized the changes in macro-dynamics of the heart associated with the transition.

Key words: ventricular fibrillation, ventricular tachycardia, VT/VF transition, calcium channels, verapamil

Introduction

Ventricular fibrillation (VF) and reentrant ventricular tachycardia (VT) are the major immediate causes of sudden cardiac death (1). Whereas VT is considered a rapid but well organized process, VF has been described as turbulent cardiac electrical activity, resulting from the random and aperiodic propagation of multiple independent wavelets throughout the cardiac muscle (2). Clinical studies have shown that VF is almost always preceded by VT of variable duration, from a few to many beats (3,4). Also, in isolated rat hearts, spontaneous transition from VT to VF is frequently observed during reperfusion after global ischemia (5). However, the manner in which VT converts to VF, or vice versa is not yet fully understood despite more than a century of intensive study.

A number of previous studies have revealed that intracellular Ca^{2+} overload predisposes the myocardium to abnormal electrical activities promoting VF (6,7). In addition, several Ca^{2+} channel antagonists have been shown to prevent VF induced by myocardial ischemia in animals (8,9), as well as to reduce cardiac mortality in patients recovering from a myocardial infarction (10,11). Merillat *et al*. (12) reported that an increase in cytosolic, global Ca^{2+} per se seems unnecessary for the initiation and maintenance of VF, and that an increase of Ca^{2+} influx

through L-type Ca^{2+} channel (slow Ca^{2+} channel) is essential. A previous report by Watanabe and Uchida (13) demonstrated in isolated rabbit hearts that rapid ventricular stimulation in the presence of verapamil, an antagonist of L-type Ca^{2+} channel, resulted in the induction of sustained monomorphic VT, not VF. In a recent study by Samie et al. (14), verapamil perfusion in Langendorff-perfused rabbit hearts converts burst pacing-induced VF to VT. They postulated that verapamil-induced VF-to-VT conversion is most likely due to a reduction in the frequency rotors and a decrease in wavefront fragmentation that lessens fibrillatory propagation. We have recently demonstrated that perfusion of isolated hearts with ruthenium red (RR), a blocker of mitochondrial Ca^{2+} uptake, reversibly converts sustained VF to VT, and that such RR-induced conversion of VF to VT is antagonized by co-treatment with S(-)-Bay K8644, an activator of L-type Ca^{2+} channels, suggesting that the inactivation of L-type Ca^{2+} channels is responsible for the RR-induced effect on the macro-dynamics of hearts (15). All these findings have led to an idea that the change in the activity of L-type Ca^{2+} channels is crucially involved in the transition between VT and VF. However, there still remains uncertainly as to what mechanisms are critically involved in the verapamil-induced VF-to-VT conversion. In addition, the changes in the macro-dynamics of hearts during

verapamil-induced VF-to-VT conversion have not yet been well characterized.

In this study, using isolated rat hearts, we investigated whether and how perfusion of the heart with verapamil, a blocker of L-type calcium channels, changed the macro-dynamics of the heart between VT and VF. This study has revealed that verapamil perfusion consistently converts rapid pacing-induced VF to VT in isolated rat hearts, and characterized such verapamil-induced changes in the macro-dynamics of the heart.

Materials and Methods

The animal experiments conformed to the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1996), as well as the “guide for the care and use of laboratory animals”, Hokkaido University School of Medicine.

Preparation of isolated heart

The method of heart isolation was described elsewhere in detail (5,15). In short, a total of 18 Male Wistar rats (8 weeks old, 240 – 260 g) were anesthetized with diethylether and administered heparin at 400 U/Kg intravenously. The chest was opened, the aorta cannulated, and the heart excised and immediately placed in ice-cold Krebs-Henseleit bicarbonate (KHB) buffer. The heart was then connected to the Langendorff apparatus and aortically perfused in a non-recirculating constant pressure (90 cmH_2O) mode. The perfusate was KHB buffer containing (in mM) NaCl 120, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.2, and glucose 11. The buffer solution was equilibrated to a 95 % O_2/5 % CO_2 gas mixture of pH 7.4 at 37℃. The heart was enclosed in a small waterjacketed chamber. The temperature of the perfusate, as well as of the atmosphere surrounding the heart, was thermostatically controlled.

A water-filled elastic balloon was inserted into the left ventricle via the left atrium. The left ventricular pressure (LVP) was monitored by a pressure transducer (DT-XXED, Ohmeda, Madison, WI) connected to the balloon, and was continuously monitored and recorded. The initial value of the end diastolic pressure (EDP) was set to 7-10 mmHg by adjusting the volume of the balloon. Unipolar left ventricular cardiomyogram (LVCMG) recordings were made by implanting enamel-insulated copper wires (diameter, 70 μm) into the left ventricular muscle. Unipolar LVCMGs were then fed to the band-pass filter (0.08 Hz – 3.0 KHz).

Induction of ventricular tachyarrhythmias

Hearts were perfused for 20 min with KHB solution (equilibrium period). The left ventricle was then electrically stimulated with a bipolar electrode inserted into the left ventricular muscle. Burst pacing (pulse interval, 40-70 ms (14-25 pulses/sec); pulse width, 1 ms; Intensity, 60-80 V) of the heart for 60 sec or more successfully induced a sustained VF or VT in most of the tested hearts (16 out of 18 hearts). Burst pacing usually induced a sustained VF, but in some cases a sustained VT. When burst pacing with the same pulse interval led to a non-sustained VF or VT, the pulse interval was automatically decreased stepwise in 5 ms

decrements from 70 or 60 ms to 40 ms (programmed electrical stimulation protocol). The duration of each step was set at 20-30 sec.

Identification of arrhythmia

A digital storage-type oscilloscope (VC-11, Nihon Kohden, Tokyo, Japan) and a thermal pen recorder were used in the identification and analysis of waveforms and the diagnosis of arrhythmia. According to Merillat *et al*. (12), VF was defined as 1) the development of a chaotic, irregular rapid LVCMG, 2) the loss of pulsatile left ventricular pressure (LVP), and 3) the loss of grossly observable, regular ventricular contraction. Monomorphic VT was defined as a rapid LVCMG with a regular and stable rhythm and a regular cyclic change in LVP with small amplitude. The induced VT or VF was considered sustained if these conditions persisted longer than 5 min.

Signal processing

The LVCMG signals stored on a DAT recorder were fed into an IBM compatible personal computer with “QuickVu-II” software (Teac, Tokyo, Japan), and analyzed on “Matlab” (The

MathWorks, Inc., MA). Spectral analysis was performed using the fast Fourier transform (FFT). Unipolar LVCMGs and LVP, in some cases, were used for the FFT analysis.

Drugs used and their application

Verapamil and nifedipine, blockers of L-type calcium channels, were obtained from Sigma (St. Louis, MO). The other chemicals were from Wako Chem. (Tokyo, Japan).

Statistics

The data are expressed as the mean ± S.D. Comparisons were performed using the one-way analysis of variance (ANOVA) followed by a paired t-Test. A *P* value of less than 0.05 was considered statistically significant.

Results

During pacing-induced sustained VF, we perfused the hearts with 600 nM verapamil, a blocker of L-type Ca^{2+} channels. Figure 1 shows an example of LVCMG, LVP, and the FFT spectrum from one heart treated with 600 nM verapamil. The LVCMG before RR perfusion was irregular (1A & 1a), with a characteristic VF-like wide-band spectrum (1d). The amplitude of LVP was small and irregular (1A & 1a). Verapamil perfusion converted the VF to VT, as is seen from the change in the pattern of the LVCMG (1A & 1b), and from the change in the FFT spectrum (1e). After the washout of verapamil, neither the LVCMG nor FFT spectrum returned to those seen before the start of verapamil perfusion during this observation period of about 3 min (1c & 1f). A short-term Fourier transformation of LVCMG indicates the characteristic changes in the frequency spectrum with time (1B). Perfusion of hearts with 200, 600 nM or 1.0 μM similarly converted the rapid pacing-induced persisting VF to VT in all of the 8 tested hearts. Figure 1C shows a short-term Fourier transformation of LVP, reflecting the characteristic changes in the frequency spectrum with time similar to that of LVCMG. Perfusion of the heart with 1.0 μM nifedipine, a different blocker of L-type Ca^{2+} channels, also converted the pacing-induced sustained VF to VT (n = 2 hearts; data not shown), suggesting that

the verapamil-induced conversion of sustained VF to VT was due to the blocking of L-type Ca^{2+} channels by verapamil.

We then performed a cross-correlation analysis between LVCMG and LVP associated with verapamil-induced conversion of pacing-induced VF to VT. Figure 2 shows an example of the analysis. During pacing-induced sustained VF, both LVCMG and LVP showed irregular and chaotic patterns (2A1), and the cross-correlation coefficient between LVCMG and LVP was small (2B1). Verapamil (600 nM) perfusion converted VF-like irregular patterns to VT-like regular ones (2A2), and the coefficient became large (2B2). The VT-like regular patterns of LVCMG and LVP persisted even after the washout of verapamil (2A3), and the correlation remained high (2B3). Figure 2C shows the change in the peak-to-peak amplitude of the correlation coefficient between LVCMG and LVP with time. The correlation suddenly became high at about 40 s after the start of verapamil perfusion. This abrupt change in the correlation was associated with the transition from VF-like to VT-like patterns in LVCMG and LVP caused by verapamil perfusion. Figure 2D shows the statistical comparison between the correlation coefficient during verapamil-induced VT and that before the treatment (VF). The coefficient during VT was significantly larger than that during VF.

Rapid pacing-induced persisting VF was often unstable, during which spontaneous transition to VT sometimes occurred (5). Therefore, the possibility that verapamil-induced conversion of pacing-induced VF to VT was due to an accidental spontaneous transition triggered by exchanging perfusion solution cannot be perfectly excluded. We thus investigated whether perfusion of hearts with verapamil resulted in the conversion of VF-like chaotic changes of LVP to VT-like regular patterns during rapid pacing (n=2 hearts). Rapid pacing of the heart with pulses of 40 ms intervals (25 pulses/s) consistently produced the stable VF-like chaotic changes in the amplitude of LVP for more than 5 min as is seen from changes in the LVP (Fig. 3A1) and their short-term Fourier transformations (3B1). During rapid pacing of the heart, 600 nM verapamil was perfused for about 70 s (3A2 & 3B2). Verapamil markedly reduced the amplitude of changes in the LVP, and gradually converted VF-like chaotic changes in the LVP to VT-like regular patterns as is seen from the change in the LVP (3g, 3h & 3i) as well as from the FFT spectrum (3j, 3k & 3l). Analysis on LVCMG was not possible because of the contamination of large stimulation artifacts in the unipolar recording of LVCMG. Expanded time records showed that changes in the amplitude of LVP were almost independent of the onset of stimulating pulses of 40 ms intervals (25 pulses/s) in the absence of verapamil

(3d-3f & 3g). During and after the perfusion of verapamil, only the spectrum component at 25 Hz became evident, although the power spectral density was low (3k, 3l). A short-term Fourier transformation of LVP also indicates the characteristic changes in the frequency spectrum with time (3B2). This result suggested that verapamil-induced conversion of VF to VT observed in Fig. 1 was not due to an accidental spontaneous transition.

We then investigated whether rapid pacing could induce sustained VF when the heart was pre-perfused with verapamil for longer durations before the application of pacing stimulus (n=2 hearts) (Fig. 4). After 1 μM verapamil perfusion for 2 min, the heart was then rapidly paced with pulses of 40 ms interval for 1 min (4A). Rapid pacing produced a sustained VT instead of VF after the end of the pacing stimulation (4A, 4a & 4b). Figures 4c and 4d shows the characteristic change of LVP response to the stimulation pulse during the continued rapid pacing. The cyclic change of LVP was phase-locked to the stimulation pulse; that is, a 1:2 entrainment occurred (4C). In contrast, the LVP change was not phase-locked to the stimulation pulses (4d). The abrupt transition in the peak frequency in the FFT spectrum from 12.5 to about 18 Hz reflects this phenomenon (4e & 4f), suggesting that the reentrant circuit independent of the stimuli was built at the time of this transition. During sustained VT

observed after the end of pacing in the presence of verapamil, the peak frequency of about 18 Hz remained almost constant. However, the peak frequency gradually became low after the washout of verapamil (4h). A short-term Fourier transformation of LVP also indicates the characteristic change in the frequency spectrum of LVP during rapid pacing as well as during and after verapamil perfusion (4B).

We then investigated whether perfusion with verapamil during pacing-induced sustained VT produced any change in either the LVCMG or the LVP (n=4 hearts) (Fig. 5). During pacing-induced sustained VT, perfusion of hearts with 1 μ M verapamil did not result in appreciable changes in the VT-like patterns of the LVCMG (5A, 5a-5c), the FFT spectrum(5d-5f), the short-term Fourier transformation of the LVCMG (5B), or the FFT spectrum (5g-5i) and the short-term Fourier transformation of the LVP (5C). This finding suggests that the activity of L-type Ca^{2+} channels was already being suppressed during sustained VT. However, 1 μ M verapamil treatment produced a gradual reduction in the peak-to-peak amplitude of the VT-like regular cyclic change in the LVP (5A & 5a-5c).

Discussion

The present study has demonstrated that perfusion of isolated hearts with either verapamil or nifedipine, blockers of L-type Ca^{2+} channels, consistently resulted in the conversion of rapid pacing-induced sustained VF to VT, and characterized the changes in macro-dynamics of the heart associated with the transition from VF to VT.

Merillat et al. (12) reported that an increase in cytosolic, global Ca^{2+} per se seems unnecessary for the initiation and maintenance of VF, and that an increase of Ca^{2+} influx through L-type Ca^{2+} channel (slow Ca^{2+} channel) is essential. Many studies supporting the idea that changes in the activity of L-type Ca^{2+} channel are involved in the initiation and maintenance of VF have been published. Rapid ventricular stimulation in isolated rabbit hearts in the presence of verapamil, a blocker of L-type Ca^{2+} channels, results in the induction of a sustained VT, not VF (13). In addition, verapamil infusion abolishes ischemia/reperfusion-induced VF in isolated rat hearts (16). Verapamil also induces a gradual transition from burst pacing-induced VF toward stationary reentrant VT reversibly (14,17). In addition, the flattening of the action potential duration (APD) restitution curve caused by the inhibition of L-type Ca^{2+} channel activity by verapamil has been shown to convert VF to VT in

canine or porcine ventricles (18,19). The slope of APD restitution is known to determine certain dynamical behavior of the heart that is relevant to the transition between VT and VF (20). All these previous findings lead to the idea that changes in the Ca^{2+} entry through the L-type Ca^{2+} channel plays a critical role in the transition between VT and VF.

Verapamil, one of the class IV antiarrhythmic agents, is not selective for the myocardial L-type Ca^{2+} channel (16). Therefore, there is a possibility that verapamil-induced conversion of pacing-induced sustained VF to VT was caused by the other drug actions of verapamil. Drug actions of verapamil include alpha receptor blockade, sodium channel blocking, recruitment of collateral flow and bradycardia (20). However, Farkas et al. (16) concluded that verapamil's protective effects on ischemia-induced VF in conscious rats (22,23) are mirrored by similar actions in Langendorff-perfused rat hearts, and appear to be mediated by blocking L-type Ca^{2+} channel. The present result that perfusion with nifedipine, a different blocker of L-type Ca^{2+} channels, also resulted in the conversion of sustained VF to VT (data not shown) seems to support this idea.

This study demonstrated that verapamil or nifedipine perfusion converted the rapid pacing-induced sustained VF to VT, suggesting that the activation of L-type Ca^{2+} channels is

involved in the transition from VT to VF. Perfusion of isolated hearts with verapamil during pacing-induced sustained VT did not result in the any appreciable change in the VT-like regular patterns of LVCMG (Fig. 5). This result suggested that the activity of L-type Ca^{2+} channels was already being suppressed during VT, and the activation of L-type Ca^{2+} channels would be necessary for the transition from VT to VF. If so, then these findings have raised the question as to what mechanisms are responsible for the activation of L-type Ca^{2+} channels associated with the transition from VT to VF.

A recent ultrastructual study (24) has revealed that Ca^{2+} release in cardiac myocytes occurs preferentially in close proximity to mitochondria; the distance between the nearest sarcoplasmic reticulum (SR) T tubule junctions and mitochondria averages 37 nm. In addition, Gathercole *et al*. (25) have shown that L-type Ca^{2+} channels are clustered in the surface plasma membrane overlying the junctional SR to secure the Ca^{2+}-induced Ca^{2+}-release (CICR) in cardiac myocytes. All these findings lead to the idea that the intracellular Ca^{2+} in the microdomains surrounding the mouth of L-type Ca^{2+} channels, SR and mitochondria would change due to the alteration of uptake of Ca^{2+} by SR and/or mitochondria, resulting in the changes of intracellular Ca^{2+} dynamics. It is well known that the activity of L-type Ca^{2+}

channels is negatively regulated by an increase in intracellular Ca^{2+} (Ca^{2+}-dependent inactivation of L-type Ca^{2+} channels) (26). These possibilities are now being investigated.

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Figure Legends

Fig. 1

Verapamil-induced conversion of pacing-induced sustained VF to VT. Perfusion of hearts with 600 nM verapamil, a blocker of L-type Ca^{2+} channels, converted the pacing-induced sustained VF to VT reversibly (A). Figures a, b, and c in the lower panel in A show the expanded recordings indicated by a, b, and c in the upper panel of LVCMG, respectively. The LVCMG with a VF-like unstable pattern (a) changed to a VT-like stable LVCMG (b) caused by verapamil treatment. The LVP with a VF-like irregular pattern (a) also changed to a VT-like regular pattern (b). The period of verapamil perfusion is indicated by a bold bar under the recording of LVP in A. Figures d, e, and f indicate the power spectrum change of LVCMG during verapamil-induced conversion of tachyarrhythmia from VF to VT. An FFT analysis was performed on the LVCMG shown in A to characterize the changes in the frequency spectrum associated with the VF to VT conversion. d, e, f: FFTanalysis on LVCMG for 9 sec during the period indicated by bold bars (d, e, f) in B. B: short-term Fourier transformation of LVCMG every 1.5 sec. Power spectral density (PSD) increases from dark blue to red through

yellow. Figures g, h, and i show the power spectrum change of LVP. g, h, i: FFTanalysis on LVP for 9 sec during the period indicated by bold bars (g, h, i) in C. C: short-term Fourier transformation of LVP every 1.5 sec. Power spectral density (PSD) increases from dark blue to red through yellow. Abbreviations: LVCMG, unipolar recording of left ventricular cardiomyogram; LVP, left ventricular pressure; PSD, power spectrum density.

Fig. 2

Changes in the cross-correlation function between LVCMG and LVP associated with verapamil-induced conversion of VF to VT. Figures A1, A2, and A3 show the LVCMG and LVP during before (A1), during (A2), and after (A3) verapamil (600 nM) perfusion, respectively. Figures B1, B2, and B3 show the cross-correlation coefficient between LVCMG and LVP before (B1), during (B2), and after (B3) verapamil perfusion, respectively. Figure C shows the changes in the cross-correlation coefficient between LVCMG and LVP with time. In this figure, the coefficient was calculated as follows: (mean peak-to-peak amplitude of the coefficient)/2.0. Figure D shows the statistical comparison between the correlation coefficient during verapamil-induced VT and that before the treatment (VF). Data are

expressed as the mean+SD (n=4 hearts for VT or VF). * $p<0.05$.

Fig. 3

Verapamil-induced conversion of VF-like irregular change in the LVP to VT-like regular cyclic change during rapid pacing. The isolated heart was paced with repetitive pulses of 40 ms interval (25 pulses/s) for more than 5 min. The upper trace of A1 indicates the pacing-induced change in the LVP. During rapid pacing of the heart, the contamination of large stimulation artifacts made it difficult to perform an FFT analysis on the unipolar LVCMG recordings. Figures a-c show the expanded time records indicated by downward arrows (a, b, c) in the LVP recording. Figures d-f show the power spectrum of the LVP for 9 sec during the periods indicated by bold bars (d, e, f) in B1. Figure B1 shows a short-term Fourier transformation of the LVP during rapid pacing. Note that rapid pacing produced stable VF-like chaotic changes in the amplitude of LVP. Figures A2 and B2 indicate the verapamil-induced change in the LVP and the short-term Fourier transformation of it during rapid pacing, respectively. The isolated heart was paced with repetitive pulses of 40 ms interval, and then perfused with 1.0 μM verapamil. Figures g, h, and i show the expanded time recordings

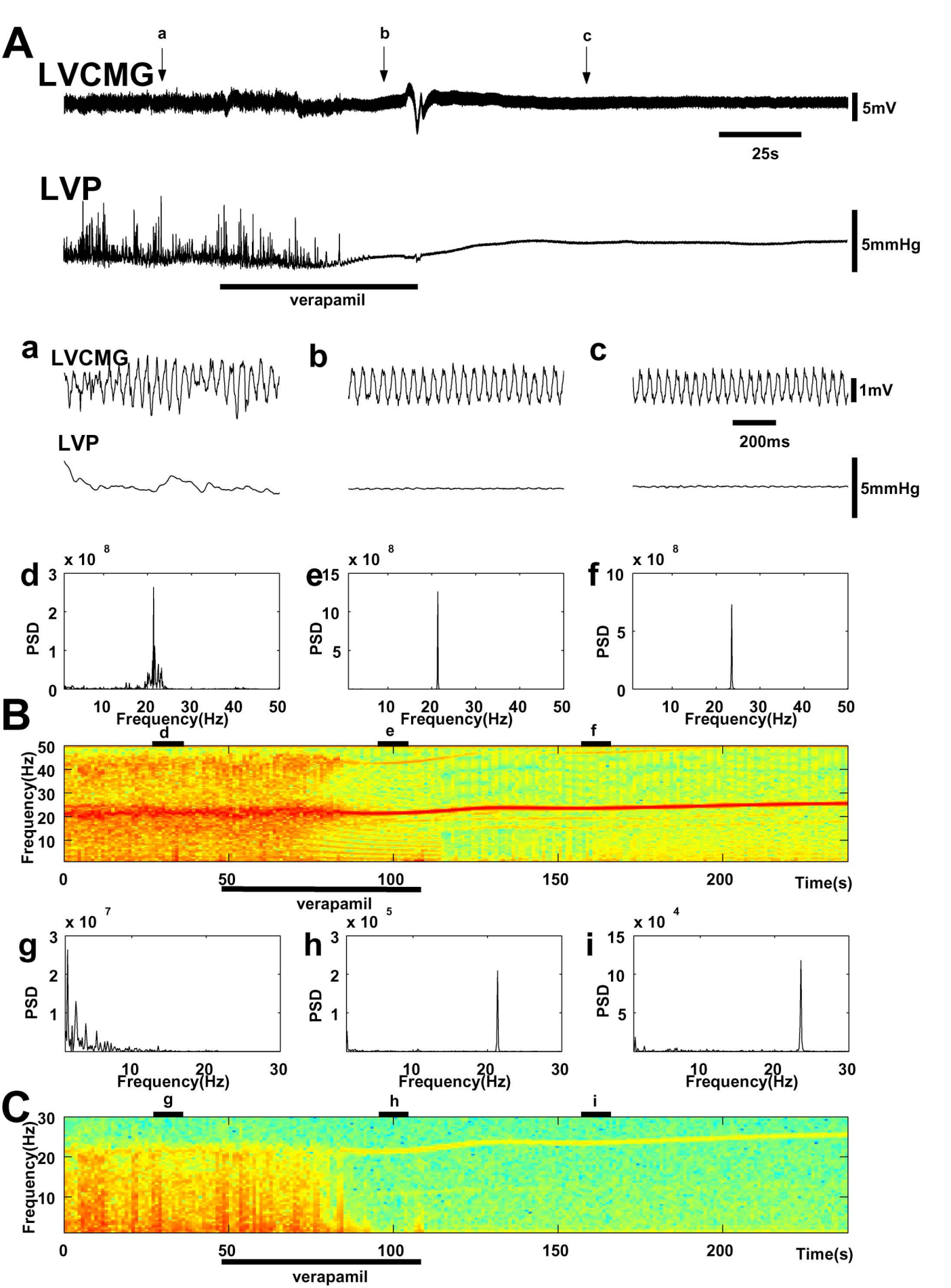
during the periods indicated by downward arrows (g, h, i) in the LVP recording. Figures j, k, and l show the power spectrum for 9 sec during the periods indicated by bold bars (j, k, l) in B2. Abbreviations: stim, pulses of rapid pacing. Other abbreviations are the same as in Fig. 1.

Fig. 4

Rapid pacing-induced sustained VT caused by pre-treatment with verapamil. After 2 min verapamil (1.0 μ M) perfusion, the heart was then paced with repetitive pulses with 40 ms interval. The rapid pacing evoked sustained VT as is seen from the LVCMG and LVP (A & a). After the washout of verapamil, the peak-to-peak amplitude of LVP gradually became larger, and the cycle period became longer (b). Figures e, f, g, and h indicate the power spectrum change of LVP. An FFT analysis was performed on the LVCMG shown in A to characterize the changes in the frequency spectrum. e, f, g, h: FFT analysis on LVP for 9 sec during the period indicated by bold bars (e, f, g, h) in B. B: short-term Fourier transformation of LVP every 1.5 sec. Power spectral density (PSD) increases from dark blue to red through yellow. See text for details.

Fig. 5

Verapamil perfusion during pacing-induced sustained VT did not produce any appreciable change in the pattern of LVCMG. During rapid pacing-induced sustained VT, 1 μM verapamil was perfused for about 2 min. Verapamil perfusion did not result in the any marked change in the LVCMG pattern, but reduced the amplitude of the regular cyclic change in the LVP (A, a, b, & c). Figures a, b, and c show the expanded time records indicated by downward arrows (a, b, c) in the LVCMG recording. Figures d, e, and f show the power spectrum of the LVCMG, also indicating that verapamil perfusion does not produce any marked change in the spectrum. d, e, f: FFT analysis on LVCMG for 9 sec during the period indicated by bold bars (d, e, f) in B. B: short-term Fourier transformation of LVCMG every 1.5 sec. Power spectral density (PSD) increases from dark blue to red through yellow. Figures g, h, and i show the power spectrum of the LVP, indicating that verapamil perfusion does not produce any marked change in the spectrum. g, h, i: FFT analysis on LVP for 9 sec during the period indicated by bold bars (g, h, i) in C. C: short-term Fourier transformation of LVP every 1.5 sec. Power spectral density (PSD) increases from dark blue to red through yellow.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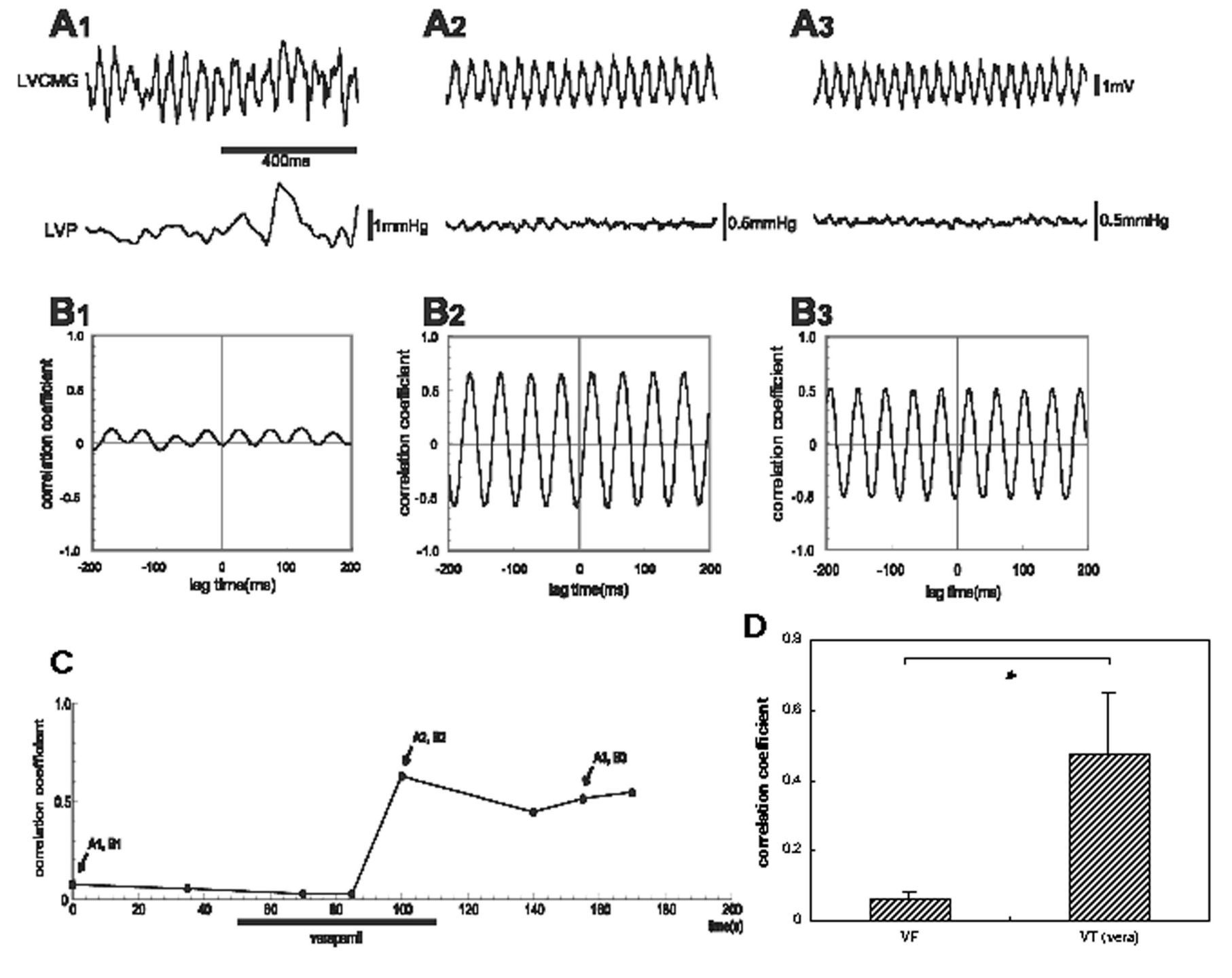
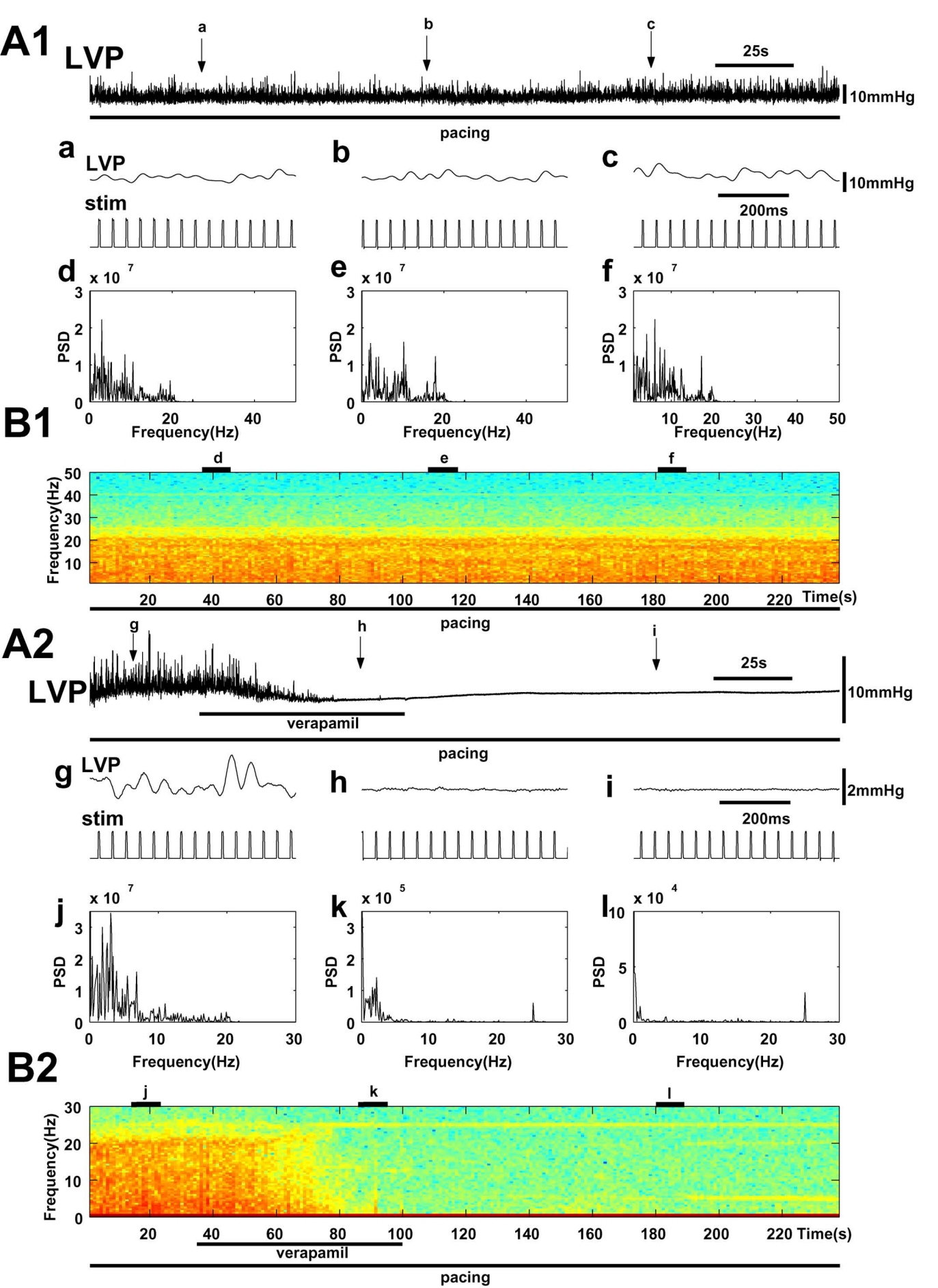
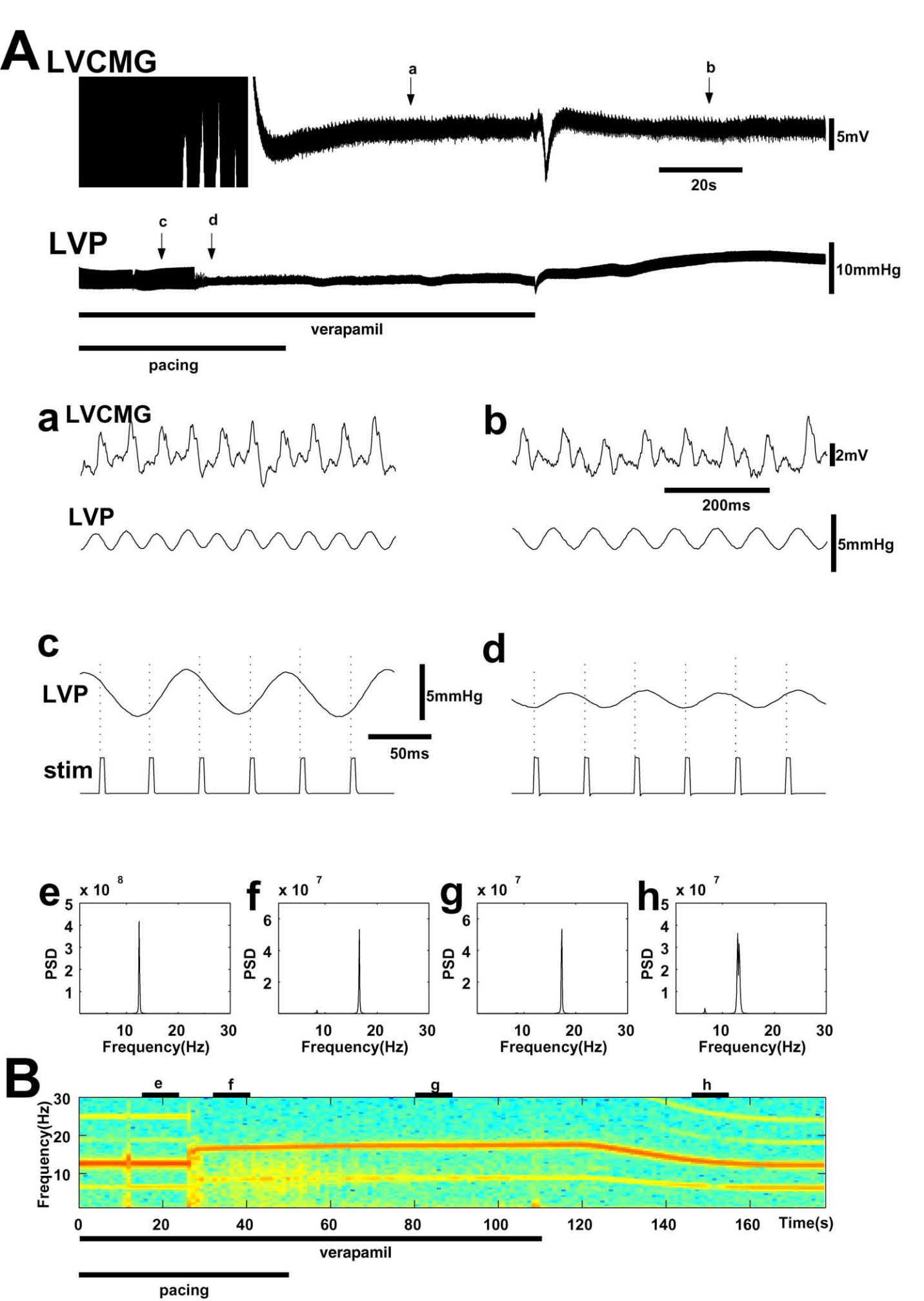
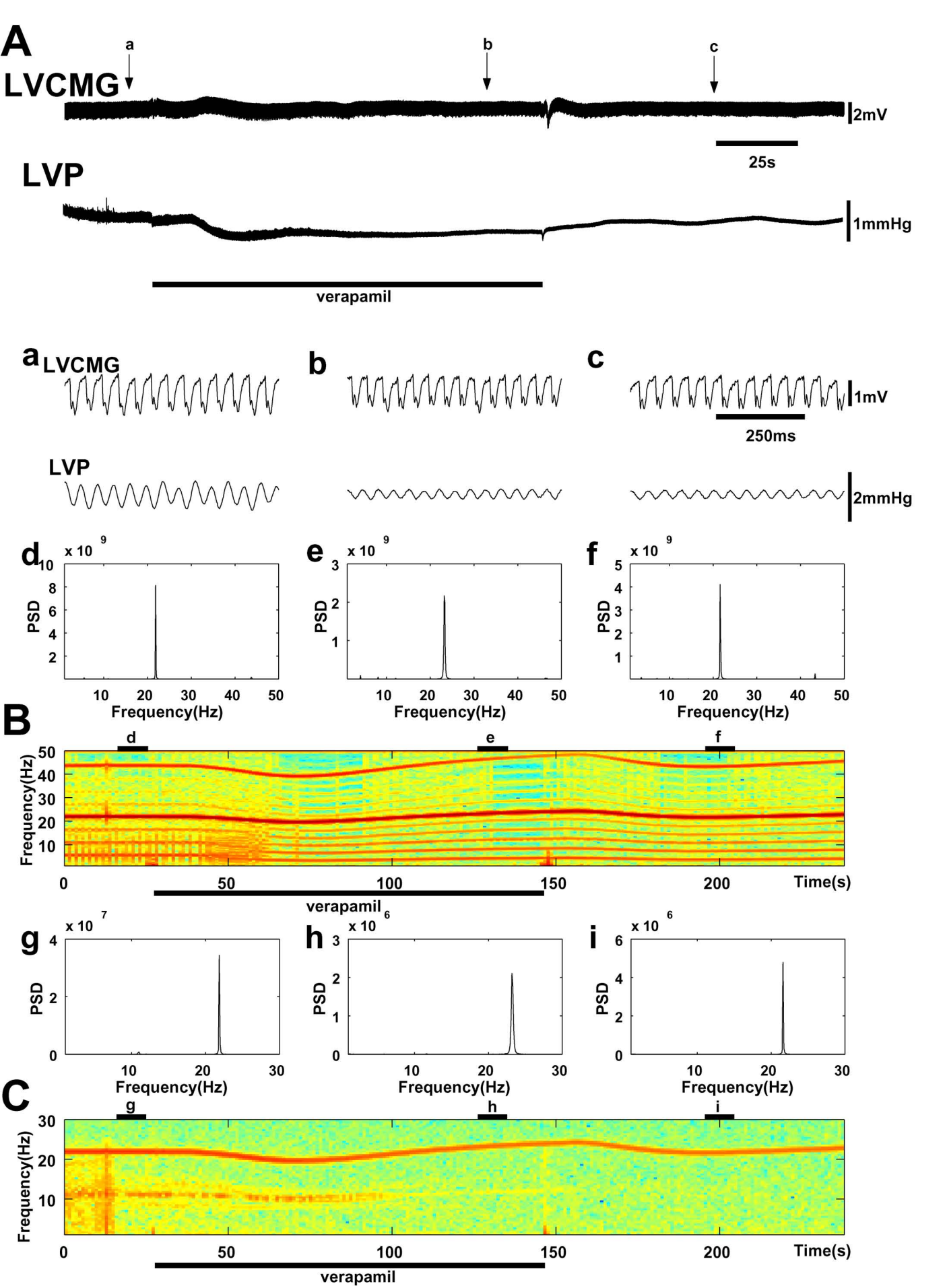
verapamil
Time(s)


A1
A2
A3
LVCMG
1mV
400ms
LVP
1mmHg
0.5mmHg
0.5mmHg
B1
B2
B3
correlation coefficient
lag time(ms)
C
A1, B1
A2, B2
A3, B3
verapamil
time(s)
D
correlation coefficient
VF
VT (vera)
*


A1
LVP
a
b
c
25s
10mmHg
pacing
a
LVP
stim
b
c
10mmHg
200ms
d
e
f
x 10 7
PSD
Frequency(Hz)
B1
d
e
f
Frequency(Hz)
Time(s)
pacing
A2
g
h
i
LVP
25s
10mmHg
verapamil
pacing
g
LVP
stim
h
i
2mmHg
200ms
j
k
l
x 10 7
x 10 5
x 10 4
PSD
Frequency(Hz)
B2
j
k
l
Frequency(Hz)
Time(s)
verapamil
pacing


A
LVCMG
a
b
5mV
20s
LVP
c
d
10mmHg
verapamil
pacing
a
LVCMG
LVP
b
2mV
200ms
5mmHg
c
LVP
5mmHg
50ms
stim
d
e
x 10 8
PSD
Frequency(Hz)
f
x 10 7
PSD
Frequency(Hz)
g
x 10 7
PSD
Frequency(Hz)
h
x 10 7
PSD
Frequency(Hz)
B
e
f
g
h
Frequency(Hz)
Time(s)
verapamil
pacing


A
a
b
c
LVCMG
2mV
25s
LVP
1mmHg
verapamil
a LVCMG
b
c
1mV
250ms
LVP
2mmHg
d
x 10 9
PSD
Frequency(Hz)
e
x 10 9
PSD
Frequency(Hz)
f
x 10 9
PSD
Frequency(Hz)
B
d
e
f
Frequency(Hz)
verapamil
Time(s)
g
x 10 7
PSD
Frequency(Hz)
h
x 10 6
PSD
Frequency(Hz)
i
x 10 6
PSD
Frequency(Hz)
C
g
h
i
Frequency(Hz)
verapamil
Time(s)