

Implications of ventricular arrhythmia vulnerability during murine electrophysiology studies

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Maguire, Colin T., Hiroko Wakimoto, Vickas V. Patel, Peter E. Hammer, Kimberlee Gauvreau, and Charles I. Berul. Implications of ventricular arrhythmia vulnerability during murine electrophysiology studies. *Physiol Genomics* 15: 84–91, 2003. First published July 29, 2003; 10.1152/physiolgenomics.00034.2003.—Programmed ventricular stimulation is being performed for the provocation of ventricular arrhythmias in genetically engineered mice. Despite the high level of interest in this area of translational research, little attention has been given to differentiating between selectivity and specificity of induced ventricular tachycardia (VT) in phenotypically normal mice. We aimed to assess factors that may enhance inducibility of VT in wild-type (WT) mice. In vivo intracardiac electrophysiological studies (EPS) were performed in 230 WT mice of 4 strains. An octapolar electrode catheter was inserted into a jugular vein and advanced to the right atrium and ventricle. Baseline ventricular conduction, refractoriness, and arrhythmia inducibility were assessed using programmed electrical stimulation (PES) and burst pacing. We found that nonsustained VT (≥ 4 beats) was inducible in 68/230 (30%) mice. Duration of VT was 1.6 ± 2.4 s, and the longest episode lasted 24 s. VT inducibility differed by strain and age. Ventricular effective refractory period (VERP) was shorter in mice with inducible VT (44 ± 12 ms) compared with noninducible mice (61 ± 16 ms, $P < 0.001$). VERP increased with age ($P < 0.001$), albeit with strain-related variability. We conclude that nonsustained VT in WT mice is reproducibly inducible and common. Genetic background variability may predispose certain strains to a higher incidence of arrhythmia induction. EPS methods impact prevalence and specificity of inducible VT. Increased VT inducibility was seen with shorter coupling intervals and application of tightly coupled extrastimuli techniques. These factors should be carefully considered when analyzing PES and burst pacing data in murine models to minimize false positives and optimize accuracy.

mice; ventricular tachycardia; programmed stimulation; genetics

MURINE MODELS FOR THE STUDY of cardiac arrhythmias have made steady gains in usage and basic research applicability. These models enable mechanistic studies

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that expose phenotypes including electrophysiological abnormalities and arrhythmias. In vivo mouse electrophysiology studies (EPS) are being performed to evaluate arrhythmia vulnerability and electrophysiological phenotypes of genetically manipulated mice (5, 8, 18). Standardized pacing and programmed electrical stimulation (PES) protocols are routinely used for EPS in mice to gauge the relative inducibility of ventricular arrhythmias. Genetics and molecular biological laboratories have a need for determination of which genotypes have an inherently greater susceptibility to arrhythmias such as ventricular tachycardia (VT). Several specific disease models have exhibited disproportionately higher rates of inducible VT compared with wild-type mice during EPS testing (6, 9, 28, 31). Until recently, induction of VT in wild-type mice had been considered to be a relatively rare event. To evaluate pacing-induced ventricular arrhythmia vulnerability in mouse models of human diseases, it will be necessary to accurately interpret the EPS findings and determine the sensitivity and specificity of murine pacing and PES protocols.

As with humans, VT can be induced by PES in structurally and electrically normal murine hearts. However, the distinction between what is normal and abnormal in terms of VT inducibility has not yet been clearly substantiated (1, 12, 13). The susceptibility to inducible VT may be influenced by a variety of factors in addition to genotype, including age, sex, strain, and autonomic and catecholaminergic state. The aims of the present study were to assess the incidence of VT encountered during standardized in vivo EPS protocols and to analyze what specific variables may enhance inducibility of ventricular tachyarrhythmias under baseline conditions in wild-type mice. We sought to determine the prevalence of inducible ventricular arrhythmia vulnerability using murine in vivo electrophysiological testing protocols.

MATERIALS AND METHODS

Animals. Surface ECGs and EPS were performed in 230 wild-type mice, compiled from control groups in prior studies. Four distinct strains were considered for analysis in this study population (129, Black Swiss, C57BL/6, and FVB). They were either inbred or had mixed genetic backgrounds. All mice were at least F₅ generation. All animal care protocols conformed to the Association for the Assessment and

Accreditation of Laboratory Animal Care, with approvals from the Children's Hospital Animal Care and Use Committee.

Procedural preparation. Protocols for the in vivo mouse electrophysiology study have been previously described in detail (5, 8, 18, 43). All mice in this study underwent identical EPS protocols. Mice were anesthetized by intraperitoneal administration of pentobarbital (0.033 mg/g). Bupivacaine (0.25%) was infiltrated subcutaneously for local anesthesia. Surface frontal-plane 6-lead ECG recordings (I, II, III, aVF, aVL, aVR) were obtained using 25-gauge electrodes placed subcutaneously in each limb. A cut-down of the right internal jugular vein was performed, and a 2-French octapolar catheter with an interelectrode interval of 0.5 mm (CIBer mouse EP; NuMed, Hopkinton, NY) was inserted. The catheter was advanced to the right atrium and ventricle using electrogram guidance and pacing capture to verify intracardiac position. Mice that died prior to completion or within 1 h of the procedure were excluded from analysis of electrophysiological parameters.

Electrophysiology study. Standard pacing protocols were used to assess ventricular conduction, refractoriness, and arrhythmia inducibility (18, 19, 39). Specifically, ventricular burst pacing was performed to evaluate the longest retrograde 1:1 VA conduction (VAC) and VA Wenckebach conduction (VAWBCL). The paced cycle length was sequentially reduced in 5-ms decrement steps with a minimal coupling interval of 50 ms. Single extrastimulation was used to determine tissue refractoriness. This test was performed at three pacing drive rates of 150, 120, and 100 ms. A drive train of eight paced beats ($S_1 \times 8$) followed by delivery of a single extrastimuli (S_2) was given until ventricular stimuli failed to result in ventricular depolarization. In an attempt to induce VT, double and triple extrastimulation techniques and burst pacing (BP) were utilized. Double and triple extrastimuli were delivered with S_2 , S_3 , or S_4 extrastimuli brought down to a minimum coupling interval of 30 ms. Ventricular burst pacing was performed as eight 50-ms and four 30-ms cycle length trains applied once every 3 s, up to a maximum 1-min time limit of total stimulation. Ventricular premature depolarizations (VPD) were defined as 1–3 beats and VT as ≥ 4 consecutive beats.

Data acquisition. Surface ECG channels were filtered between 0.5 and 400 Hz. Intracardiac electrograms were amplified and filtered between 5 and 400 Hz, at an acquisition rate of 2,000 samples per second. Surface ECG and electrogram signals were displayed on an oscilloscope and simultaneously recorded through an analog-to-digital converter (AD Instruments, Grand Junction, CO) for offline analysis. All surface ECG measurements [sinus cycle length (SCL), PR, QRS, and QT] were performed manually with online calipers. Specifically, the QRS interval was measured from the initial positive or negative deflection to the major rapid down stroke, and the QT interval was measured from the onset of the QRS complex to the end of the T wave (The point at which the voltage returns to the isoelectric baseline). Three consecutive beats were measured and averaged by two independent observers.

VT cycle length analysis. VT episodes were initially documented during EPS and visually reconfirmed as a part of the offline analysis. With the use of PowerLab software (AD Instruments), segments of VT (≥ 4 beats) were identified and manually extracted from stored data files for further characterization (317 episodes). Subsequently, these digital recordings were transferred to a customized software program written in Matlab (Mathworks, Natick, MA). The total duration of each pacing-induced arrhythmia was calculated (in seconds). The autocorrelation functions of both the surface

ECG and intracardiac signal were computed, and the location of the maximum value of these functions between 30 and 100 ms from zero-lag was chosen as the tachycardia cycle length (TCL). If TCL computed from both the ECG and the intracardiac signal agreed (within $\pm 10\%$), then the value was accepted, but if not, both signals along with their autocorrelation functions were presented for manual selection of TCL (19).

Statistical analysis. Values of continuous variables are presented as means ± 1 SD. Surface ECG intervals were measured in six-limb leads by two independent observers and averaged for analysis. Means were compared across subgroups using the two-sample *t*-test for two groups and the one-way analysis of variance for three or more groups. If differences among means were detected using analysis of variance, then Scheffé's subgroup testing for multiple comparisons was performed. Proportions of mice inducible by programmed ventricular stimulation were compared across subgroups using Fisher's exact test. Analysis of interobserver variability was performed where appropriate. $P < 0.05$ was considered to be statistically significant.

RESULTS

From an initial cohort of 255 wild-type mice, 230 (90%) underwent full in vivo electrophysiological examination, while 25 mice were excluded due to death prior to or within 1 h of study completion. None of the excluded animals was observed to have VT prior to death. In total, 68 of 230 mice (30%) were inducible by programmed ventricular stimulation. In these 68 mice, 317 episodes of VT were observed in all. PES alone induced 115 episodes in 31 mice (Fig. 1). Burst ventricular pacing was more aggressive and increased the sensitivity nearly twofold; VT was induced in 61 mice with 202 episodes. Ease of reproducibility varied and each animal had the possibility of being induced during PES alone, BP alone, or both modes. Therefore, in some cases, mice could be induced during PES and then again for a second time during BP ($n = 30$, 44%). Arrhythmia induction technique yielded a predictable stepwise increase in VT inducibility (singles 3%, doubles 6%, triples 11%, and burst pacing 27%). The influences of sex, age, and strain on the surface ECG parameters are summarized in Table 1. The PR, QT, and QTc values in the youngest mice (≤ 8 wk old) tended to be shorter than in older mice. These demographic variables are analyzed with respect to ventricular electrophysiological parameters in Table 2. As a whole, there were no significant sex differences in any of the ECG measurements, ventricular refractoriness, or retrograde ventriculo-atrial conduction. A subgroup analysis of age and strain on ventricular arrhythmia vulnerability is presented as a 6×4 contingency table (Table 3). Reliability coefficients between observers ranged from 0.88–0.95 for all ECG intervals and electrophysiological parameters measured.

The duration of each episode of VT averaged 1.6 ± 2.4 s, ranging from 1 to 24 s (Fig. 2). The vast majority of induced VT episodes lasted less than 10 s (Tables 4 and 5), particularly with programmed stimulation. However, the cycle length of VT was typically shorter when induced with programmed stimulation compared

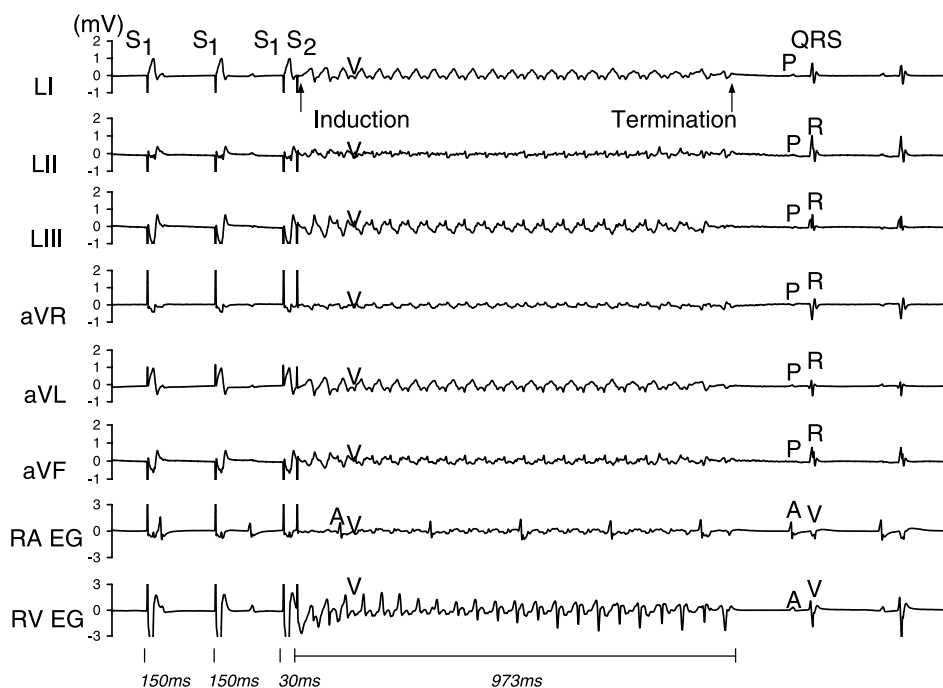


Fig. 1. A typical run of pacing-induced ventricular tachycardia (VT) in a wild-type mouse. ECG leads I, II, III, aVR, aVL, aVF, and intracardiac electrograms (right atrial RA EG and right ventricular RV EG) are displayed from top to bottom. A drive train of eight paced ventricular beats at 150 ms ($S_1 \times 8$; last three shown) is followed by a single ventricular extrastimulus (S_2), delivered at a coupling interval of 30 ms. This induces non-sustained monomorphic VT, which spontaneously terminates and converts back into sinus rhythm. A, atrial electrogram; V, ventricular electrogram; ordinate is in mV; abscissa is in seconds.

with those episodes induced with burst ventricular pacing (48 ± 8 vs. 63 ± 9 ms). The induced VT episodes varied with regard to morphology and VA association (Fig. 3). Some episodes appeared monomorphic ($n = 114$, 36%), while others had a more polymorphic appearance ($n = 203$, 64%). VT inducibility values of inbred (26%) and hybrid (37%) mice were not statistically different overall. However, VT inducibility varied significantly on the four different genetic backgrounds ($P < 0.001$). Only a single episode of VT was induced in the C57 strain. In contrast, inducibility was higher in the other tested backgrounds, including 129 (29%), FVB (43%), and Black Swiss (35%) with VT, reflecting strain-specific vulnerabilities. Identifying a short ven-

tricular refractoriness was an accurate predictor of arrhythmia induction. Mice were paced at S_1 cycle lengths of 150, 120, and 100 ms. At each of these three coupling intervals, ventricular effective refractory period (VERP) was significantly longer in mice in which VT was noninducible (61 ± 18 , 62 ± 16 , and 58 ± 14 ms, respectively, $n = 162$; vs. 43 ± 12 , 45 ± 13 , and 44 ± 11 ms, $n = 68$, $P < 0.001$ for each comparison). In the age-related comparison, the youngest mice had significantly shorter refractory periods than the three older age groups ($P < 0.001$). Refractory periods were similar between inbred and hybrid mice. However, analysis of the individual strains revealed differences in refractoriness and retrograde VAC. The Black Swiss

Table 1. Surface ECG interval data summary

	<i>n</i>	Weight, g	SCL, ms	HR, beats/min	PR, ms	QRS, ms	QT, ms	QTc(m), ms
Total	230	30 ± 9	172 ± 27	349 ± 55	39 ± 5	14 ± 2	34 ± 5	26 ± 5
Noninducible	162	31 ± 9	$177 \pm 28^*$	$339 \pm 49^*$	40 ± 5	14 ± 2	$36 \pm 6^\ddagger$	27 ± 6
VT	68	30 ± 10	163 ± 28	378 ± 61	39 ± 6	14 ± 2	31 ± 6	24 ± 5
Sex								
Male	151	$32 \pm 7^\ddagger$	175 ± 29	343 ± 55	40 ± 5	14 ± 2	35 ± 6	26 ± 5
Female	79	26 ± 7	167 ± 26	359 ± 58	39 ± 5	14 ± 2	33 ± 8	25 ± 6
Age								
≤ 8 wk (5 ± 2 wk)	49	$21 \pm 5^\ddagger$	169 ± 27	355 ± 53	$35 \pm 4^\ddagger$	13 ± 2	$28 \pm 6^\ddagger$	$22 \pm 5^\ddagger$
9–15 (12 ± 2 wk)	60	26 ± 5	179 ± 30	335 ± 55	40 ± 5	13 ± 2	35 ± 7	26 ± 5
16–49 (34 ± 8 wk)	51	32 ± 8	175 ± 32	343 ± 59	41 ± 5	15 ± 2	34 ± 6	26 ± 5
≥ 50 (65 ± 18 wk)	70	38 ± 7	164 ± 24	366 ± 52	41 ± 5	14 ± 2	36 ± 7	29 ± 6
Strain								
Hybrid	71	$34 \pm 11^\ddagger$	167 ± 27	366 ± 53	$41 \pm 5^*$	14 ± 2	33 ± 6	26 ± 5
Inbred	159	26 ± 6	175 ± 29	343 ± 58	39 ± 5	14 ± 2	34 ± 7	26 ± 6
129	52	27 ± 4	172 ± 29	349 ± 55	$43 \pm 4^\ddagger$	$16 \pm 2^\ddagger$	$38 \pm 6^\ddagger$	$29 \pm 5^\ddagger$
BS	26	27 ± 4	186 ± 33	323 ± 52	40 ± 3	13 ± 2	32 ± 5	24 ± 3
C57	41	28 ± 5	177 ± 25	339 ± 51	39 ± 4	13 ± 1	36 ± 4	28 ± 3
FVB	40	26 ± 9	165 ± 31	364 ± 62	34 ± 4	14 ± 2	30 ± 7	24 ± 5

Values are means \pm SE. SCL, sinus cycle length; HR, heart rate; VT, ventricular tachycardia. $*P < 0.05$ across subgroups. $^\ddagger P < 0.001$ across subgroups.

Table 2. Ventricular electrophysiological data summary

	n	VT Mice, %	VAWBCL, ms	VERP		
				150, ms	120 ms	100 ms
Total	230		135 ± 24	55 ± 18	55 ± 17	53 ± 13
Noninducible	162		139 ± 27*	61 ± 18‡	62 ± 16‡	58 ± 14‡
VT	68		129 ± 25	43 ± 12	45 ± 13	44 ± 11
Sex						
Male	151	43 (28%)	137 ± 26	55 ± 19	56 ± 17	53 ± 14
Female	79	25 (32%)	133 ± 27	53 ± 17	55 ± 17	50 ± 15
Age						
≤ 8 wk (5 ± 2 wk)	49	17 (35%)*	138 ± 22‡	41 ± 13‡	43 ± 15‡	41 ± 12‡
9–15 (12 ± 2 wk)	60	11 (18%)	155 ± 34	55 ± 16	58 ± 16	54 ± 15
16–49 (34 ± 8 wk)	51	15 (29%)	133 ± 22	64 ± 18	63 ± 19	62 ± 16
≥ 50 (65 ± 18 wk)	70	25 (36%)	125 ± 25	55 ± 19	59 ± 18	55 ± 12
Strain						
Hybrid	71	26 (37%)	132 ± 23	55 ± 20	55 ± 18	55 ± 17
Inbred	159	42 (26%)	139 ± 30	55 ± 16	56 ± 18	51 ± 13
129	52	15 (29%)‡	133 ± 24*	62 ± 16‡	61 ± 14‡	60 ± 10‡
BS	26	9 (35%)	148 ± 16	46 ± 13	48 ± 12	46 ± 11
C57	41	1 (2%)	164 ± 35	61 ± 11	73 ± 15	64 ± 9
FVB	40	17 (43%)	128 ± 24	42 ± 14	45 ± 13	44 ± 12

Values are means ± SE. VERP, ventricular effective refractory period; VAWBCL, ventriculoatrial Wenckebach cycle length; RVERP, right ventricular effective refractory period. * $P < 0.05$ across subgroups. ‡ $P < 0.001$ across subgroups

and FVB strains had significantly shorter ventricular effective refractory periods, whereas the 129 and C57 strains had comparatively longer refractory periods at all tested pacing cycle lengths. The VAWBCL values were longer for C57 strain than either 129 or FVB by analysis of variance. The particular methods of VT induction are specified in further detail in Table 6, suggesting the specificity of each of the electrophysiological tests for VT provocation using programmed stimulation and pacing.

DISCUSSION

In this study we demonstrate that in vivo catheter-based ventricular stimulation reliably induces ventricular arrhythmias in wild-type mice. Approximately one-third of all mice studied by standardized pacing and PES had inducible VT, yielding hundreds of arrhythmic events. Incidence and reproducibility of ventricular arrhythmias was strongly dependent upon specific EPS methods employed. Smaller numbers of extrastimuli and wider coupled paced beats produced far fewer inducible ventricular arrhythmias, whereas application of more aggressive extrastimulation techniques caused increases in VT frequency and duration. Our results indicate that ventricular tachyarrhythmias induced by high-frequency burst-electrical stimulation should be looked at with caution because of potential inaccuracies in selectivity and low specificity.

This may avoid misinterpretation of inducible arrhythmias in murine models of cardiac phenotyping.

As a result of a relatively high number of mice being inducible, we were able to examine multiple variables that may affect ventricular arrhythmia vulnerability. ECG analysis did not detect any apparent sex differences in the measured intervals. There was an age-related shortening in PR and QT intervals of juvenile mice (5 wk old), and there were differences observed in intervals between particular strains.

The most notable electrophysiological parameter that correlated with induction of VT was tissue refractoriness. Mice with inducible VT had significantly shorter VERPs. Refractory periods demonstrated an age dependency, with juvenile mice having significantly shorter refractory periods compared with the older age groups. There were also age- and strain-related differences in retrograde ventriculo-atrial conduction, which also correlated with VT susceptibility. The youngest (5 wk old) and oldest (over 1 yr old) mice in this study had the highest incidence of inducible VT. The short refractory periods of juvenile mice may have provided a favorable substrate for conducting tightly coupled extrastimuli. The sex comparison revealed no differences between males and females in the induction of VT. Interestingly, strain-related differences in VT inducibility were observed. The incidence of VT did not differ between hybrid and inbred mice as a whole.

Table 3. Age and strain subgroup ventricular tachycardia inducibility analysis

Age Group	Strain					
	Hybrid	Inbred	129	BS	C57	FVB
≤ 8 wk	5/14 (36%)	12/35 (34%)	0/6 (0%)	3/7 (43%)	0/5 (0%)	9/17 (53%)
9–15 wk	3/6 (50%)	8/54 (15%)	2/11 (18%)	4/9 (44%)	0/25 (0%)	2/9 (22%)
16–49 wk	5/19 (26%)	10/32 (31%)	7/17 (41%)	1/5 (20%)	0/5 (0%)	2/5 (40%)
> 50 wk	13/32 (41%)	12/38 (32%)	6/18 (33%)	1/5 (20%)	1/6 (17%)	4/9 (44%)

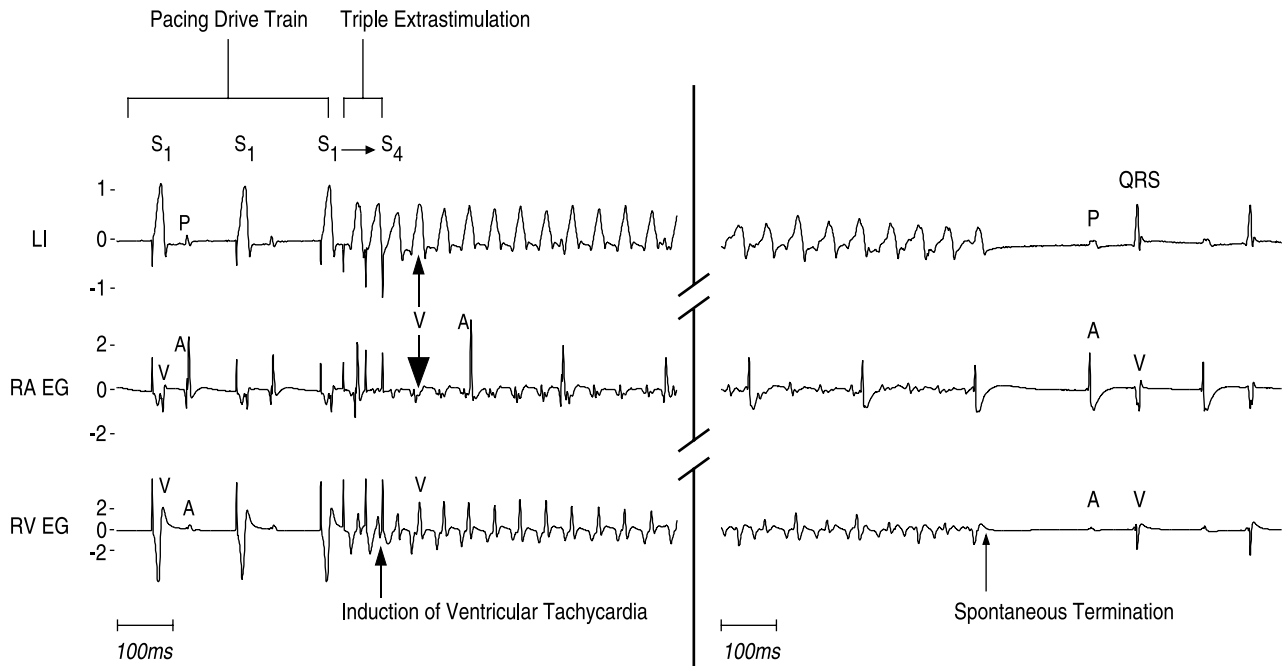


Fig. 2. Induction of sustained VT in a wild-type mouse. ECG lead I (top), right atrial intracardiac electrogram (middle), and right ventricular intracardiac electrogram (bottom) are shown from a wild-type mouse. This example shows a longer run of pacing-induced VT, which lasted for 17 s. A: programmed triple extrastimulation ($150 \times 8/40/40/30$ ms) sequence that induced sustained monomorphic VT. B: spontaneous termination of VT and conversion back into normal sinus rhythm. The pacing drive train was set at 150 ms. Ordinate is in mV; abscissa is in seconds.

However, the four different inbred strains displayed differences in the occurrence of VT. Mice on the FVB and Black Swiss backgrounds had more inducible VT, whereas the C57 mice were less susceptible to VT inducibility. It appears that strain-dependent genetic factors contribute significantly to vulnerability of ventricular tachyarrhythmias. These results therefore raise important implications of whether certain strains of mice would affect the phenotypic expression of ventricular arrhythmias that occur during in vivo EPS, limiting statistical accuracy in defining true arrhythmia susceptibility.

In summary, both monomorphic and polymorphic VT can be induced in wild-type mice using standard pacing and programmed ventricular stimulation protocols. Most induced VT episodes are short and terminate spontaneously. Characterization of inducible VT in wild-type mice is critical to the understanding and relevance of electrophysiological findings in genetically engineered mouse models.

Previous reports of VT in mice. An early study by Nwangwu et al. (35) documented the first in vivo murine VT in a single surface ECG lead for preliminary screening of antiarrhythmic agents. However, few reports were published about mouse VT until the 1990s, when, due to the scientific progress in molecular biology and genetics, relevant murine models of human diseases were engineered. These include models of inherited cardiac electrophysiological diseases such as long QT syndromes and dilated and hypertrophic cardiomyopathies (6, 32, 45). Since then, several reports have described VT in mice using isolated myocardial fibers (46), ex vivo whole heart preparations (4, 14, 36), and in vivo EPS (7, 11, 24, 30, 38). London et al. (32) recorded spontaneous VT by implantable telemetry devices in conscious long QT mice. In 2,000, Nguyen-Tran et al. (34) elegantly presented ECG recordings of sudden cardiac death using telemetry techniques. In vivo inducible VT was demonstrated in some of the genetically engineered models including familial hypertrophic cardiomyopathy mice (9, 10), long QT syn-

Table 4. VT episodes organized by duration

	4–9b	10b–1sec	1–2 s	2–10 s	>10 s	Total
PES						
Episodes (mice)	39(18)	43(16)	20(11)	12(6)	1(1)	115(31)
BP						
Episodes (mice)	97(42)	38(21)	26(16)	36(24)	5(3)	202(61)

PES, programmed electrical stimulation; BP, burst pacing. * $P < 0.05$

Table 5. VT episode characteristics

	VT Cycle Length, ms	Duration, s
PES	$48 \pm 8^*$	1.2 ± 1.9
BP	63 ± 9	1.9 ± 2.5
PES and BP	57 ± 8	1.6 ± 2.4

* $P < 0.05$.

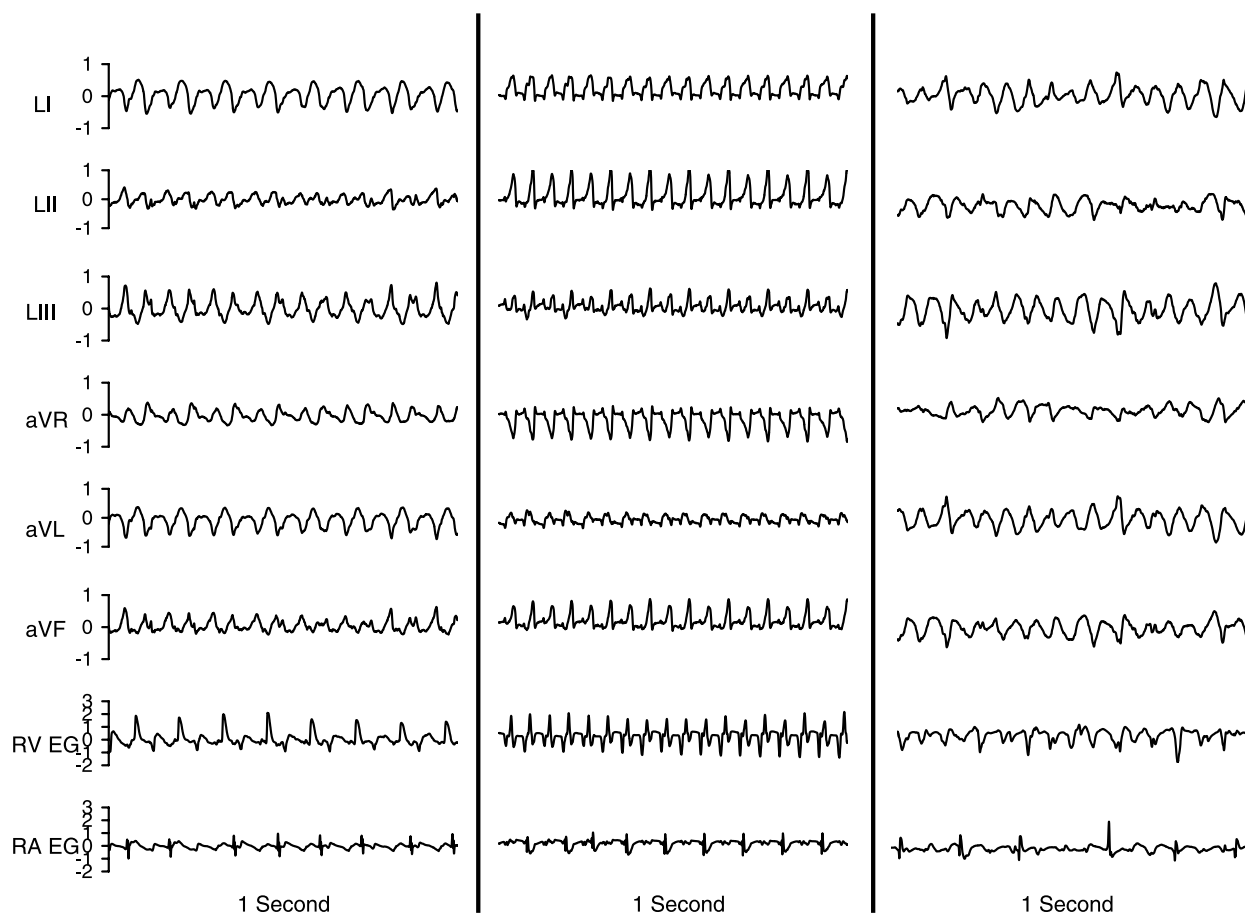


Fig. 3. VT morphologies in three wild-type mice. ECG leads I, II, III, aVR, aVL, aVF, and intracardiac electrograms (right ventricular RV EG and right atrial RA EG) are shown from *top to bottom*. Displayed from *left to right* are examples of right axis deviation VT, normal axis monomorphic VT, and polymorphic VT.

drome mice (23, 24, 30), connexin gene defects (11, 15, 27, 31, 42), and in murine models of myocardial infarction (20, 28). Mouse models of human familial diseases causing VT, ionic channel defects, or other factors that may contribute in VT induction continue to increase. Therefore, it is imperative to determine the statistical accuracy of electrophysiological tests, the important nuances that differ between strains and EPS protocols, and the relevant implications of inducible arrhythmias in genetically engineered mice.

False-positive VT in human PES studies. Electrophysiology testing is utilized in humans for determination of VT vulnerability and risk of sudden cardiac death, yet remains fraught with false-positive and false-negative results. PES appears useful for reproducing reentrant type VTs but is less helpful in identifying or excluding automatic ventricular rhythms (1,

12, 13, 47). In addition, the sensitivity and specificity of PES in humans varies depending on the electrical and structural substrates. For example, electrophysiology studies have been demonstrated to be informative in patients with repaired congenital heart disease or bundle branch reentry VT induction (2, 26, 37, 41) but less useful for prediction of VT vulnerability in patients with severe ventricular dysfunction such as dilated cardiomyopathies or electrical disorders such as long QT syndromes (3, 16, 17, 22, 25, 29, 40).

Limitations. In general, induced arrhythmias during electrophysiological testing may be dissimilar from the mechanisms occurring during spontaneous cardiac arrhythmias or sudden death. Future refinements in the acquisition of conduction velocity, ventricular refractoriness, and action potential duration data in intact mice may supplement information regarding arrhyth-

Table 6. Method of VT induction

	Singles: ($S_1 \times 8$) + S_2	Doubles: ($S_1 \times 8$) + S_2S_3	Triples: ($S_1 \times 8$) + $S_2S_3S_4$	Coupling Interval ≥ 50 ms	Coupling Interval < 50 ms	Pacing: (S_1) ≥ 50 ms	Burst Pacing: 8×50 ms; 4×30 ms
Mice	8 (3%)	14 (6%)	25 (11%)	3 (1%)	30 (13%)	15 (7%)	61 (27%)
Episode	15	31	69	13	102	38	164

mia wavelength and other mechanistic features (21, 33, 44). Finally, mouse models of ventricular tachyarrhythmia and miniaturization of catheter-based in vivo EPS methodologies may not be relevantly extrapolated to clinical disease pathophysiology or electrophysiological risk stratification in humans.

Conclusions. Nonsustained VT in wild-type mice is reproducibly inducible and not uncommon. Genetic background variability may predispose certain strains to a higher incidence of arrhythmia induction. EPS induction methods have a significant impact on prevalence of inducible ventricular arrhythmias in wild-type mice. There was a significant stepwise increase in VT inducibility with shorter coupling intervals and application of aggressively coupled extrastimuli techniques. These factors should be carefully considered when analyzing PES and burst pacing data in murine models to minimize false positives and optimize accuracy. Caution is suggested in the interpretation of inducible arrhythmias in mouse models of genetic diseases.

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