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Pathogenesis of Lethal Cardiac Arrhythmias in *Mecp2* Mutant Mice: Implication for Therapy in Rett Syndrome

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Abstract

Rett Syndrome is a neurodevelopmental disorder typically caused by mutations in *Methyl-CpG-Binding Protein 2 (MECP2)* in which 26% of deaths are sudden and of unknown cause. To explore the hypothesis that these deaths may be due to cardiac dysfunction, we characterized the electrocardiograms (ECGs) in 379 people with Rett syndrome and found that 18.5% show prolongation of the corrected QT interval (QTc), indicating a repolarization abnormality that can predispose to the development of an unstable fatal cardiac rhythm. Male mice lacking MeCP2 function, *Mecp2*^{Null/Y}, also have prolonged QTc and show increased susceptibility to induced ventricular tachycardia. Female heterozygous null mice, *Mecp2*^{Null/+}, show an age-dependent prolongation of QTc associated with ventricular tachycardia and cardiac-related death. Genetic deletion of MeCP2 function in only the nervous system was sufficient to cause long QTc and ventricular tachycardia, implicating neuronally-mediated changes to cardiac electrical conduction as a potential cause of ventricular tachycardia in Rett syndrome. The standard therapy for prolonged QTc in Rett syndrome, β -adrenergic receptor blockers, did not prevent ventricular

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tachycardia in *Mecp2^{Null/Y}* mice. To determine whether an alternative therapy would be more appropriate, we characterized cardiomyocytes from *Mecp2^{Null/Y}* mice and found increased persistent sodium current, which was normalized when cells were treated with the sodium channel-blocking anti-seizure drug phenytoin. Treatment with phenytoin reduced both QTc and sustained ventricular tachycardia in *Mecp2^{Null/Y}* mice. These results demonstrate that cardiac abnormalities in Rett syndrome are secondary to abnormal nervous system control, which leads to increased persistent sodium current. Our findings suggest that treatment in people with Rett syndrome would be more effective if it targeted the increased persistent sodium current in order to prevent lethal cardiac arrhythmias.

Keywords

Rett Syndrome; autonomic nervous system; arrhythmia; ventricular tachycardia; QT interval

INTRODUCTION

Rett syndrome (RTT, MIM #312750) is a severe X-linked dominant neurodevelopmental disorder that typically affects girls and is characterized by regression of spoken language, loss of hand use, problems with ambulation, and development of repetitive hand stereotypies (1). RTT is typically caused by mutations in *Methyl-CpG-Binding Protein 2 (MECP2)* (2), a gene encoding a protein involved in regulation of gene expression (3). In addition to the cognitive and motor abnormalities present in RTT, affected people also show autonomic dysfunction, with breathing and heart rate control irregularities (4–7). Boys with mutations in *MECP2* exhibit more severe autonomic dysfunction with marked breathing and heart rate abnormalities that result in death within the first year of life (8).

People with RTT have a high incidence of sudden unexpected deaths (26% of all deaths) (9), which are believed to have a cardiac origin. Previous work has indicated that some people with RTT have prolonged QT intervals (LQT) on electrocardiograms (ECGs) (10). LQT has been shown in other patient populations to be a significant risk factor for sudden arrhythmic death (11). To date, the cause for LQT in RTT is unknown, and its contribution to the high proportion of sudden death is yet untested.

The most common causes of inherited LQT include mutations in the voltage-gated potassium channels *KVLQT1* (LQT1) and *HERG* (LQT2), and in the voltage-gated sodium channel *SCN5A* (LQT3), although rare mutations in genes encoding other channel subunits and in other cardiac proteins such as caveolin-3 (12) may also contribute in a minority of inherited cases (13). In addition to genetic causes, alterations in autonomic nervous system function have been shown to alter cardiac repolarization and contribute to the long QT phenotype (14). Patients with potassium channelopathies (LQT1–2) respond to β -adrenergic receptor blockade as an effective anti-arrhythmic prophylactic therapy; in contrast, LQT patients with sodium channelopathies (LQT3) have a poor response to β -adrenergic receptor blockade in the prevention of arrhythmias, indicating that two different mechanisms apply in how sodium and potassium channelopathies contribute to LQTs (11).

Given that RTT patients have MeCP2 dysfunction leading to LQT phenotype, we sought to define whether: (i) MeCP2 dysfunction in mice recapitulates the long QT phenotype and causes predisposition to arrhythmic-induced death after programmed electrical stimulation (ii) MeCP2 dysfunction in neuronal tissue alone is sufficient to reproduce the LQT phenotype, and (iii) whether alterations in the sodium current may underlie a significant portion of the LQT phenotype in this mouse model of RTT.

RESULTS

Long QTc is common in people with RTT and reproduced in the animal model of RTT

To define the prevalence of electrophysiological abnormalities in people with RTT, we evaluated ECGs from 379 girls and women with typical RTT enrolled in the Rett Syndrome Natural History Study. Seventy people (18.5%) had QTc over 450ms, the established threshold for LQT (Figure 1A–B), consistent with previous small-scale studies (10, 15, 16). As 26% of deaths in RTT are sudden and unexpected (11), these 18.5% of affected individuals are likely at risk for sudden death.

To determine the origin of LQT in RTT, we sought to identify electrophysiological abnormalities in mouse models of RTT. Because human boys with mutations in *MECP2* are more severely affected than girls, with severe autonomic dysfunction and early death (17), we first characterized male mice lacking MeCP2 at 2–3 month of age, the time-point at which the animals manifest the most severe motor and behavioral phenotypes. Using surface ECG, we found that male mice hemizygous for *Mecp2* (*Mecp2*^{Null/Y}) exhibited longer QTc intervals than WT mice (Figure 1C–E). Moreover, QRS intervals in *Mecp2*^{Null/Y} were longer than those in WT mice (Figure 1F), but none of the other electrical properties that we recorded (RR, PQ, QRS, QT, QTc, SCL, AV intervals) were different between the two animal groups (Table 1). To determine whether this repolarization abnormality represented by the prolonged QTc interval was associated with any contractility or structural deficit in the hearts of *Mecp2*^{Null/Y} animals, we performed echocardiography and found no evidence of any structural or contractility abnormality in *Mecp2*^{Null/Y} animals compared with control animals (Table 2).

We next sought to determine whether the propensity toward LQT found in male hemizygous mice is also present in mice that precisely mimicked RTT, females heterozygous for a null allele of *Mecp2*. 4–5 month-old female heterozygous mice (*Mecp2*^{Null/+}) did not show a significantly longer QTc interval or changes in QRS duration (Figure 1G, H). However, older 9–10 month-old female *Mecp2*^{Null/+} mice demonstrated LQT (Figure 1G) and prolonged QRS (Figure 1H), with no change in other ECG parameters (Table 3). As in the male animals, echocardiography did not reveal structural heart abnormalities or contractility deficits in female *Mecp2*^{Null/+} mice (Table 4). Thus, hemizygous male *Mecp2*^{Null/Y} mice have severe early-onset LQT and QRS prolongation, and heterozygous female *Mecp2*^{Null/+} show prolongation of both parameters that becomes apparent at older ages. A similar phenomenon is seen in patients with MeCP2 deficiency: Boys lacking MeCP2 function typically die of severe neurological disease within the first years of life (8), whereas females have relatively normal development until about 18 months of age, when they develop progressive onset of neurological regression (1).

RTT mice show increased susceptibility to induced ventricular tachycardia

Given the significant LQT in male *Mecp2*^{Null/Y} and female *Mecp2*^{Null/+} mice and the association between LQT and development of ventricular arrhythmias, we hypothesized that these mice may be more susceptible to develop ventricular arrhythmias. To test this, we inserted a catheter into the right ventricle of anesthetized mice and electrically stimulated the heart using programmed electrical stimulation (PES) to determine susceptibility towards cardiac arrhythmias. Male *Mecp2*^{Null/Y} mice developed sustained ventricular tachycardia (VT, defined as ventricular tachycardia of more than 1s) more often than did WT mice immediately following ventricular stimulation (Fig. 2A–B). The duration of any (including non-sustained) arrhythmia episodes was significantly longer in *Mecp2*^{Null/Y} mice than in WT mice (Fig. 2C). Interestingly, one of the male *Mecp2*^{Null/Y} died secondary to a cardiac arrhythmia induced during PES.

Similar to the age-dependent nature of LQT in female *Mecp2*^{Null/+} mice, young 4–5 month-old female *Mecp2*^{Null/+} mice did not develop a significant number of arrhythmias (Figure 2E–F). However, every older female *Mecp2*^{Null/+} mouse tested showed PES-induced ventricular arrhythmias, whereas none of the WT mice had PES-induced arrhythmias (Fig. 2D–E). Additionally, 29% (2/7 mice) of female *Mecp2*^{Null/+} mice died of VT during ventricular stimulation, suggesting that older female *Mecp2*^{Null/+} mice with LQT are at risk for arrhythmia-induced death (Figure 2F–G).

Cardiac abnormalities in RTT mice result from loss of MeCP2 function within the nervous system

Removing MeCP2 function from only the nervous system reproduces all the phenotypes of animals lacking MeCP2 in all tissues, including premature death (18). To determine whether loss of MeCP2 function within the nervous system would also cause LQT and increased susceptibility to ventricular arrhythmias, we generated a nervous system-specific conditional knockout (NKO) using the Nestin-Cre/loxP system, which restricts knockout of MeCP2 to the nervous system (19). *Mecp2* mRNA expression was eliminated from the brain in these NKO animals, but was unaffected in the heart (Figure 3A). As previously determined, the conditional allele of *Mecp2*, FLOX, shows a slight decrease in brain *Mecp2* mRNA expression compared with WT (20), however *Mecp2* mRNA was not significantly reduced in the hearts of FLOX compared with WT. QTc was increased in male NKO mice versus both FLOX and WT controls (Figure 3B). As expected, the hypomorphic FLOX animals showed an intermediate phenotype, with mildly prolonged QTc duration, however the NKO animals were significantly increased over these FLOX controls. This LQT was associated with an increased incidence of VT in male NKO versus WT controls (Figure 3C) after PES, whereas the FLOX animals were not different from WT. The incidence of VT was higher in the NKO animals (64%) compared with the FLOX animals (25%, $P=0.09$). In addition, 2 of the 11 NKO mice that we tested died of VT after pacing, suggesting that neuronal deficiency of *Mecp2* is sufficient to cause both LQT and pacing-induced arrhythmias and arrhythmia-induced death (Figure 3D).

Alteration in sodium current underlies LQT and the susceptibility to ventricular arrhythmia in RTT mice

Because the exact etiology of LQT in RTT is poorly understood, current strategies to prevent sudden arrhythmic events in RTT have been empirical. The standard therapy to prevent arrhythmias in RTT is to treat prophylactically with a β -adrenergic receptor blocker such as propranolol and to reduce exposure to drugs that might lengthen the QTc interval, such as anticholinergic agents. We evaluated the effectiveness of treatment with a β -receptor blocker therapy in male *Mecp2*^{Null/Y} mice using PES. Heart rate (HR) decreased in WT mice after injection of propranolol (3 mg/kg i.p.), whereas *Mecp2*^{Null/Y} mice experienced a less pronounced decrease in HR (Fig. 4A). Propranolol did not reduce QTc in either male WT or *Mecp2*^{Null/Y} mice (Fig. 4B). Similarly, propranolol did not protect male *Mecp2*^{Null/Y} mice from PES-induced arrhythmias. All male *Mecp2*^{Null/Y} mice treated with propranolol experienced either VT (60%) or atrioventricular block (40%) leading to death (Fig. 4C), whereas no treated WT mice had arrhythmias. These results suggest that propranolol may not be an effective drug for the treatment of QT prolongation and arrhythmias in people with RTT.

β -adrenergic receptor blockers have been described as being efficacious primarily in LQT1 and LQT2 syndromes, which are ascribed to potassium channelopathies; however, beta-blockers have not been found to be effective anti-arrhythmic agents in primary sodium channelopathies such as LQT3 or Brugada syndrome (21). To determine whether alteration in the voltage-gated sodium channel current caused the LQT phenotype in male *Mecp2*^{Null/Y}

mice, we performed patch clamping in isolated ventricular myocytes in order to measure the voltage-gated sodium channel current from these animals. Although there was no difference in peak sodium current at -20mV (WT: -18.8468 ± 1.8873 pA/pF; *Mecp2*^{Null/Y}: -16.5563 ± 1.0314 pA/pF; $P=0.3$), and no difference in the cellular capacitance (WT: 198 ± 19 pF; *Mecp2*^{Null/Y}: 176 ± 28 pF), measurements of persistent sodium channel current (I_{Na}) showed a larger I_{Na} in *Mecp2*^{Null/Y} mice versus WT (Figure 4D–E). We similarly performed patch clamping in isolated ventricular myocytes from NKO animals and found they had an increased I_{Na} compared with the WT littermate animals of the *Mecp2*^{Null/Y} mice (Figure 4D). Although this comparison was not made to the exact littermate control animals, the persistent I_{Na} is robust within WT animals if the genetic strain is identical. In this experiment, the background genetic strain was identical between the *Mecp2*^{Null/Y} line and the NKO line.

In the genetic forms of LQT that do not respond to β -adrenergic receptor blockers, such as LQT3, drugs that target the sodium channel prevent cardiac arrhythmias (10). Given the observations that (i) the β -adrenergic receptor blocker propranolol could not alter either QTc interval or arrhythmia incidence in *Mecp2*^{Null/Y} mice, (ii) a persistent late I_{Na} current existed in *Mecp2*^{Null/Y} mice, and (iii) seizures are common in human RTT, we decided to evaluate phenytoin (PHT) as a potential therapeutic agent in RTT. PHT is a persistent late I_{Na} blocking drug, which prevents cardiac arrhythmias and neurological epileptic seizures, and thus may be able to prevent of ventricular arrhythmias in MeCP2 deficiency. When cardiomyocytes from were treated with PHT, the late I_{Na} decreased compared to untreated male *Mecp2*^{Null/Y} myocytes, demonstrating that the persistent late I_{Na} in *Mecp2*^{Null/Y} mice is reversible by pharmacological blockade of the sodium channel (Fig. 4D–E). Injection of PHT (50 mg/kg i.p) into WT and *Mecp2*^{Null/Y} mice decreased QTc to a value similar to that of PHT-treated WT mice (Fig. 4F–H) and completely abolished ventricular arrhythmias in *Mecp2*^{Null/Y} mice (Fig. 4H), indicating that I_{Na} is a significant component of arrhythmias in RTT and that PHT or drugs with similar pharmacology may reduce arrhythmia risk in people with RTT.

DISCUSSION

In this work, we utilized a large cohort of people with RTT to determine that LQT is found in nearly 20% of people with RTT. This LQT is suspected to underlie the sudden unexpected deaths found in this disease (22). We determined that mouse models of RTT have LQT and are susceptible to VT and sudden cardiac death. The development of these cardiac abnormalities occurs in an age-dependent fashion in female heterozygous animals, indicating that this is likely a secondary effect. This idea was confirmed by the surprising finding that the increased persistent I_{Na} , LQT, VT, and sudden cardiac death were the result of loss of MeCP2 function within the nervous system rather than within the cardiomyocytes themselves.

Although β -adrenergic receptor blockers are the mainstay of therapy for the majority of cases of LQT, and RTT specifically, we found that in the animal model β -adrenergic receptor blockers did not have the expected efficacy in preventing VT and may have increased arrhythmic death, similar to findings in a mouse model of LQT3 syndrome (21). Cardiomyocytes isolated from RTT mouse hearts showed an increased I_{Na} , similar to that seen in genetic forms of LQT that affect Na channels. This increased I_{Na} was blocked by treatment with a Na-channel blocker, PHT. Treatment of the mouse model with PHT shortened the QTc interval and prevented VT and cardiac death, raising the possibility that such treatments may prove effective in people with RTT.

An unexpected result of this work is the finding that the cardiac arrhythmias present in the animals are the result of changes in MeCP2 function within the nervous system. This was unexpected because LQT reflects alteration in the repolarization property of cardiomyocytes themselves, and the genetic causes of idiopathic LQT are the result of mutations in genes that encode proteins within the cardiomyocytes that control the electrical properties of those cells. In fact, we found that the electrical properties of cardiomyocytes from both *Mecp2*^{Null/Y} and NKO animals are indeed changed. Thus, the alteration of the electrical properties in the cardiomyocytes is an indirect response to alterations of the nervous system control of the heart.

Neurological dysfunction may affect the control of cardiac rate and rhythm. First, repetitive seizures can induce remodeling of the potassium and Na-channels within the heart, leading to QTc prolongation and cardiac arrhythmias (23). Second, autonomic neuropathies have previously been described to prolong QTc interval in patients with primary CNS disease (14, 24–27), autonomic neuropathy (28, 29), and amyotrophic lateral sclerosis (30). The exact mechanism by which altered nervous system control leads to cardiac arrhythmias in these cases is unknown. It has been suspected that sympathovagal imbalance in people with RTT may contribute to sudden cardiac death (10, 22), even in the presence of normal cardiac function revealed by echocardiography (31). An important question is whether this imbalance indirectly leads to QTc prolongation. Further characterization in mouse models of RTT may be helpful in understanding the mechanism underlying this prolongation, however, this characterization may be challenging because heart rate in mice is primarily controlled by sympathetic control (32).

As sympathetic over activation appears to be present in people with RTT (10), it is somewhat surprising that inhibition of the sympathetic outflow via β -adrenergic receptor blockers did not appear to be beneficial in the animal model of RTT. The poor response to β -adrenergic receptor blockers in these animals is consistent with the increased late I_{NaP} , which is reminiscent of the inability of β -adrenergic receptor blockers to suppress arrhythmias in people with LQT syndromes caused by sodium channel mutations, such as in LQT3. In LQT3, bradycardia caused by unchecked parasympathetic activation can lead to life threatening cardiac arrhythmias (21). We similarly saw sudden cardiac death after β -adrenergic receptor blockade in female *Mecp2*^{Null/+} mice, with some of the animals actually dying in atrioventricular block.

This study has the inherent limitation of using a knockout mouse to model human disease. Mice typically have shorter QT intervals than human patients, and their neurological development may favor parasympathetic control of HR, which is greater than human patients (33). Another limitation in this work is our inability to determine whether there is an age-dependent change in QTc prolongation in people with RTT. Although the data presented here is based on a very large cohort of affected people, additional information will be gained as the Rett Syndrome Natural History Study progresses and true longitudinal information gathered with repeated ECG recordings. Furthermore, it would have been beneficial to determine from the human data whether exposure to specific drugs or specific *MECP2* mutations alter the propensity towards developing QTc prolongation. Again, the ongoing natural history study will hopefully answer these questions. In this study, mice lacking MeCP2, a genetic mimic of the human condition, showed the same cardiac phenotype as people with RTT, indicating that these mice may be useful for determining the underlying pathophysiological mechanisms as well as for testing therapeutic options for RTT. We have demonstrated this latter point by showing that acute treatment of *Mecp2* null mice with PHT may protect against this disorder. This is not presently the standard of care for LQT in RTT: we recommend that alternative therapies should be tested in RTT, focusing on the use of Na-channel blocking agents. In this study, we chose PHT as a representative of a class of Na-

channel blocking agents that are commonly used in neurological diseases for their anti-seizure effects, but these drugs could also be useful for preventing cardiac arrhythmias. Future work using the cellular and animal system presented here will help determine whether PHT, other anti-epileptic drugs that block the sodium channel, or newer antiarrhythmic agents such as ranolazine that inhibit persistent I_{Na} is the best therapeutic option in this disease. Such information will then be useful to guide formal clinical trials in people with RTT.

METHODS

Human subjects

This study was approved by the institutional review boards of the participating centers. Written, informed consent was obtained from all guardians of patients participating in the study (clinicaltrials.gov, Identifier NCT00296764). The study methodology has been described (34). For this work, ECG results obtained by referring physicians were reviewed and QTc interval for the first ECG was obtained, and the age at which this test was performed was recorded.

Animals

Mecp2^{Tm1.1Bird} and *Mecp2^{Tm1Bird}* mice (35) were obtained as a gift from Dr. Adrian Bird, and experimental heterozygous *Mecp2^{Tm1.1Bird/+}* female animals were generated as described (36). To generate a nervous system knock out of MeCP2, a conditional allele of MeCP2, *Mecp2^{Tm1Bird}* (35) on a pure 129S6 background was mated to Nestin-Cre (Stock 003771 Jackson Labs) maintained on a C57Bl/6 background to generate F1 129S6.C57Bl/6 experimental animals. All animals were housed in the AAALAC approved animal facility at Baylor College of Medicine and experiments approved by the Baylor IACUC.

Transthoracic echocardiography

Mice were anesthetized with 1.5% isoflurane in 95% O₂. Body temperature was maintained at 36°C and 37°C on a heated platform, and ECGs and temperature were continuously monitored. Cardiac function was assessed with the use of a VisualSonics VeVo 770 Imaging System (VisualSonics), equipped with a high-frequency 30-MHz probe, as described (37).

Surface ECG

Mice were anesthetized with 1.5% isoflurane in 95% O₂ and six-lead ECGs recorded by pad electrodes with bandpass filtering between 0.03 Hz and 1 kHz, according to published methods (38). QT values were calculated as the interval between the onset of the QRS complex and the moment after the T-wave peak (the first derivative (dV/dt) becomes zero). Corrected QT intervals (QTc) were calculated by the formula $QTc = QT + 0.3173 \times (170 - RR)$ as described (39).

Programmed electrical stimulation

Atrial and ventricular intracardiac electrograms were recorded with the use of a 1.1F octapolar electrode catheter (EPR-800; Millar Instruments) inserted into the right ventricle via the right jugular vein, as described (40, 41). Inducibility of VT was determined using overdrive pacing and extra stimulus protocols and sustained VT is defined as VT lasting more than 1s. For acute treatment with antiarrhythmic agents, animals were injected intraperitoneally 40 minutes before catheterization with 3mg/kg of a propranolol solution or 120 minutes before with 50mg/kg of phenytoin.

RNA Isolation and Quantitative Real Time PCR

Animals were killed by cervical dislocation after Avertin anesthesia and hearts were rapidly dissected, washed in 1xPBS to remove blood, and snap frozen in liquid nitrogen. They were then homogenized in Trizol (Invitrogen) using a Polytron. RNA was extracted by per the manufacturer's instructions. cDNA was made using q Script cDNA kit (Qantas) and qPCR performed on a Biorad CFX96 qPCR machine (Biorad) using PerfeCTa SYBRGreen FastMix (Qantas) as described (20).

Patch Clamping of Ventricular Myocytes

Enzymatic isolation of ventricular myocytes from WT and *Mecp2*^{Null/Y} mice was performed by the Langendorff perfusion system as described (40). Macroscopic I_{Na} was recorded by the whole-cell patch clamp technique by Axon 200B amplifier (Axon Instruments). Briefly, isolated myocytes were perfused at room temperature with bath solution (in mM): 130 NaCl, 10 CsCl, 1 MgCl₂, 10 HEPES, 10 Glucose, 1 4-AP, pH 7.4, with CsOH. Electrodes were pulled with series resistance around 1.5M Ω and filled with pipette solution (in mM): 130 CsCl, 10 NaCl, 10 HEPES, 10 EGTA, pH 7.4, with CsOH. Recording protocols were generated with pClamp software 9 (Axon Instruments) and digitized at 25 kHz using a digidata 1332A A/D converter. To activate late I_{Na} , a 1s depolarizing step pulse to -20 mV from a holding potential of -120 mV was applied at a rate of 0.1 Hz. The magnitude of steady late I_{Na} during the 350 ms to 800 ms were calculated by integrating the current over that time period using the integration feature of Clampfit 10.0 software, as described (42). PHT was acutely provided to cardiomyocytes in solution at a final concentration of 6 μ g/mL, the cells incubated for 10 minutes, and then patch clamping performed as above.

Statistical analysis

Continuous variables were expressed as mean \pm SEM; whenever the distribution was skewed, medians with the first and third quartiles were expressed. Continuous variables were evaluated with an unpaired Student *t* test or ANOVA. The Mann-Whitney test was used to compare continuous variables with a skewed distribution. Categorical data were expressed as percentages and were compared with the Fisher exact test. *p* < 0.05 was considered statistically significant.

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ABBREVIATIONS

ECG electrocardiogram

KO	Knockout
LQT	long corrected QT interval
MECP2	Methyl-CpG-binding protein 2
NKO	Nervous-system Knockout
PES	programmed electrical stimulation
PHT	phenytoin
QRS	QRS interval
QT	QT Interval
QTc	QT Interval corrected for heart rate
RTT	Rett Syndrome
SCD	Sudden cardiac death
SVT	Sustained Ventricular Tachycardia
VF	Ventricular Fibrillation
VT	Ventricular Tachycardia

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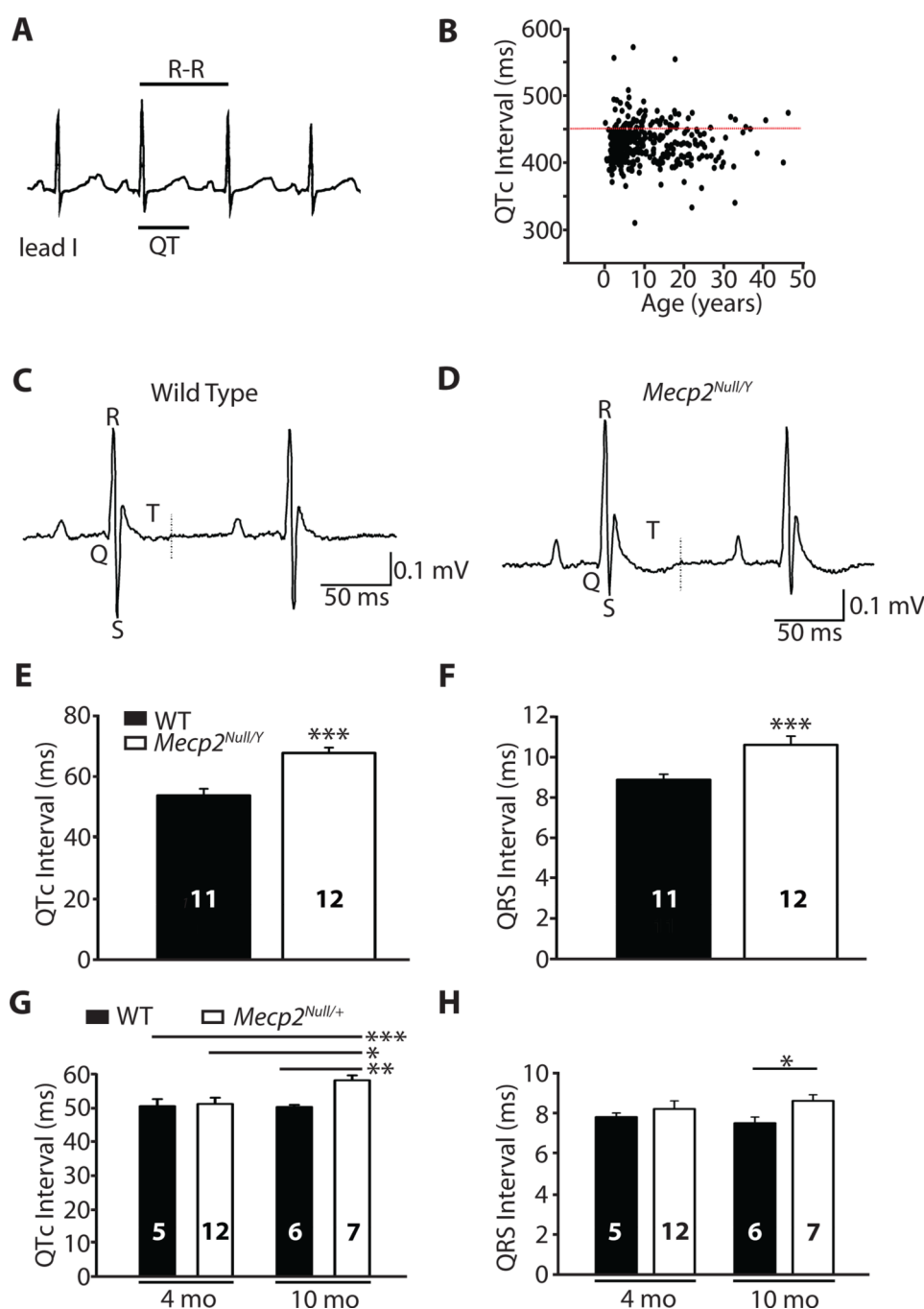


Fig. 1. *Mecp2* deficiency is associated with LQT

(A) A representative ECG from a human patient with RTT with the R-R and QT interval identified. (B) A scatter plot of QTc interval as a function of age in years for 379 RTT patients. The red dashed line indicates the cutoff (450ms) for prolonged QTc. (C) A representative ECG from a wild type (WT) mouse at baseline. (D) A representative ECG from a *Mecp2*^{Null/Y} male mouse at 2 months old. (E–H) Quantification of the ECGs show increased QTc and QRS (F) intervals in *Mecp2*^{Null/Y} mice. Young *Mecp2*^{+/-} female mice show normal QTc (G) and QRS (H). However, older female *Mecp2*^{+/-} mice show LQT (G)

and increased QRS duration (**H**). * $P<0.05$, ** $P<0.01$, *** $P<0.001$. N, number of mice as indicated within bars.

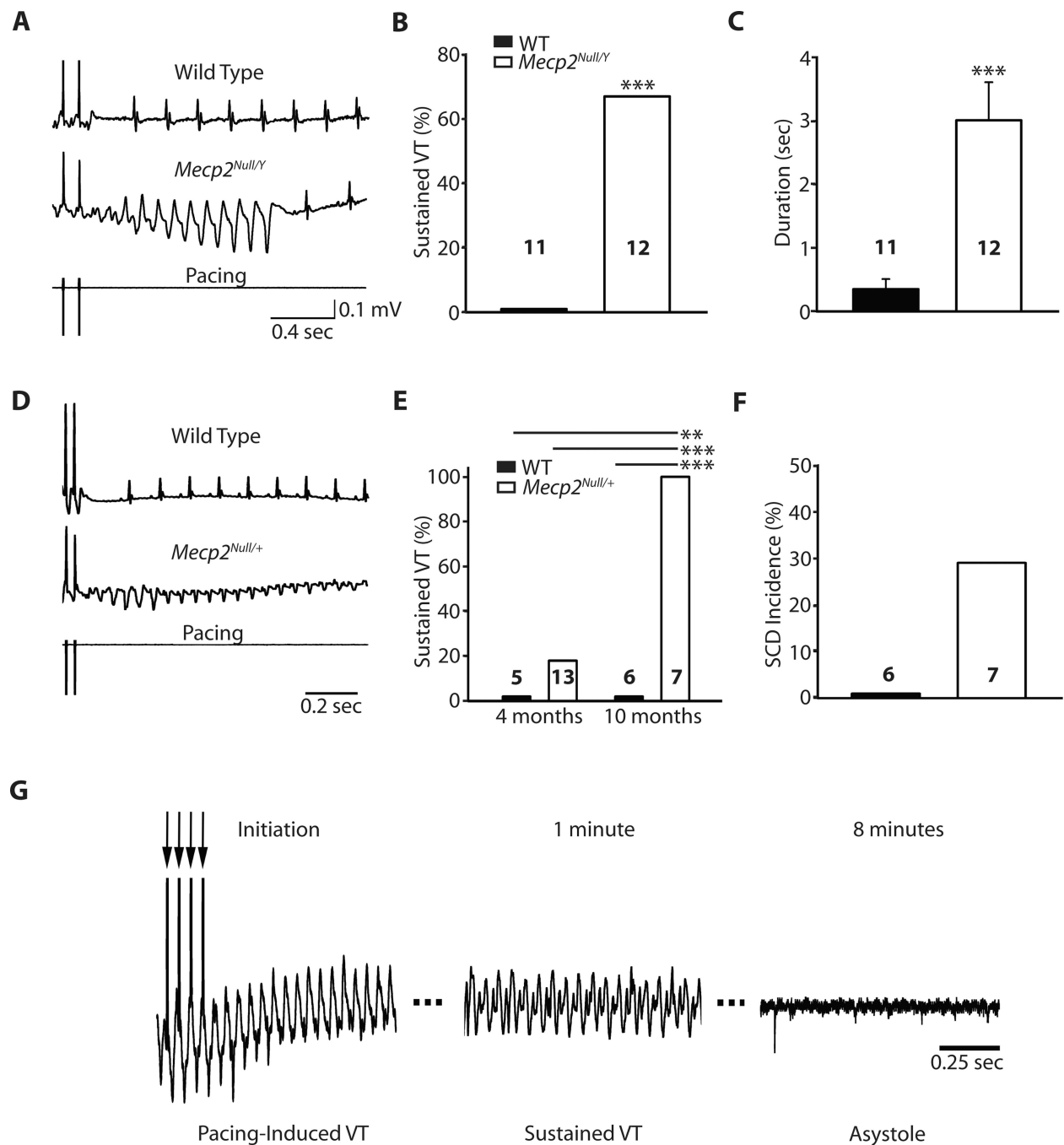


Fig. 2. *Mecp2* deficiency is associated with predisposition to induced VT and arrhythmia-induced death in mice

(A) Representative surface ECG tracings of wild type (top) and male *Mecp2*^{Null/Y} mice (middle) after pacing (bottom). (B) Incidence of sustained VT after pacing in *Mecp2*^{Null/Y} and WT mice. (C) Arrhythmia duration of any type in *Mecp2*^{Null/Y} and WT mice. (D) Representative surface ECG tracings from wild type (top) and *Mecp2*^{Null/+} mice (middle) after pacing (bottom). (E) Age-dependent susceptibility of sustained VT. (F) Incidence of sudden cardiac death (SCD) in WT and *Mecp2*^{Null/+} mice at 10 months. 2 of 7 *Mecp2*^{Null/+} mice developed ventricular tachycardia and ventricular fibrillation after intracardiac pacing, causing death. (G) ECG of a 10 months old *Mecp2*^{+/-} mouse showing development of

sustained VT leading to asystole at 8 minutes after pacing. ** $P<0.01$, *** $P<0.001$. N is indicated within the bars of the graphs.

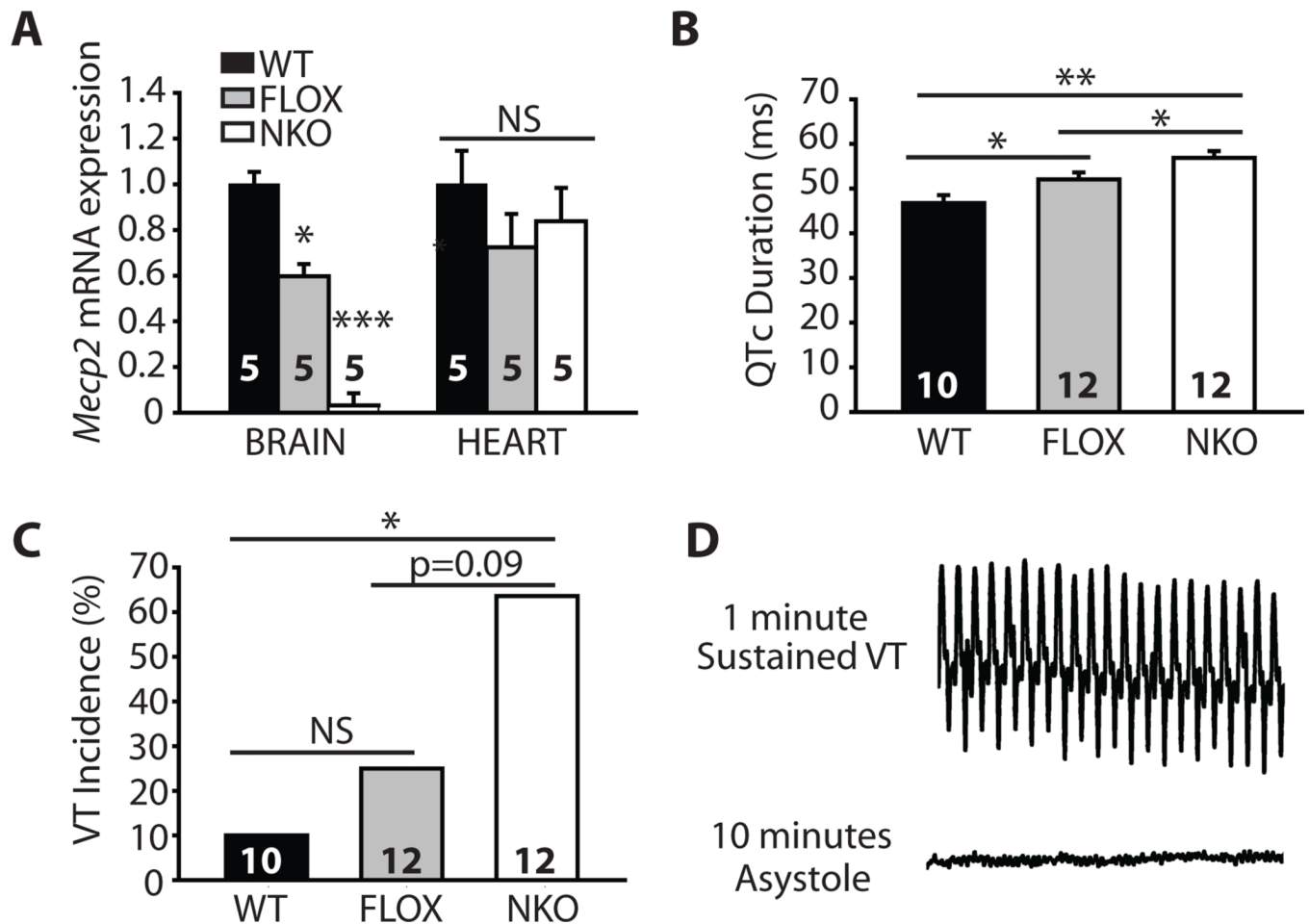


Fig. 3. Male mice with nervous system-specific conditional knockout of MeCP2 (NKO) have LQT and inducible arrhythmias

(A) *Mecp2* mRNA expression in brain and heart normalized to WT. * $P < 0.05$ FLOX compared to WT, *** $P < 0.01$ NKO compared with WT. (B) QTc duration and (C) VT incidence of WT, FLOX, and NKO male mice. * $P < 0.05$, ** $P < 0.01$. N is shown within the bars of the graphs. NS, non-significant. (D) Example of an ECG from one of two NKO mice that died of ventricular arrhythmia, showing the ECG 1 minute and 10 minutes after PES-induced VT.

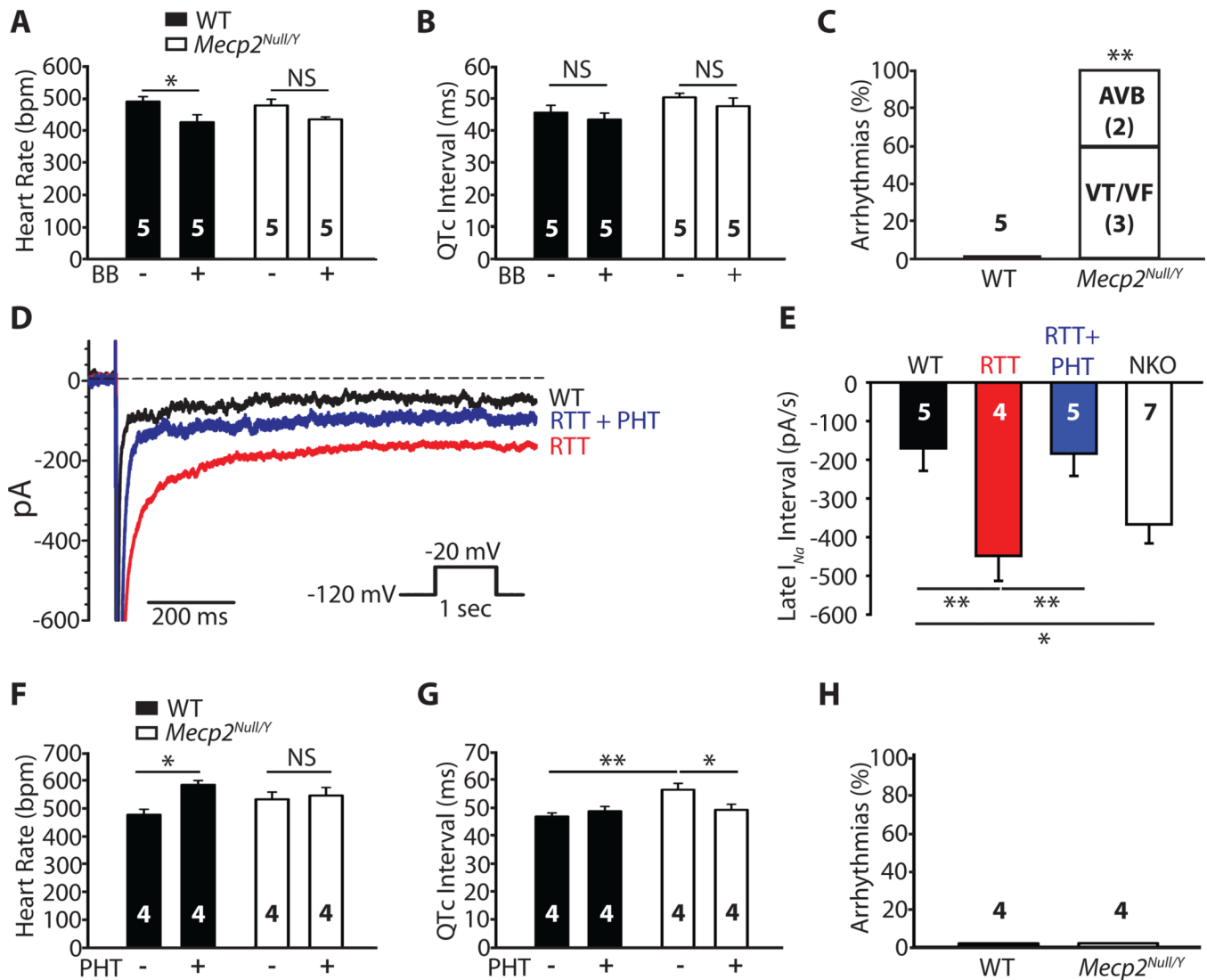


Figure 4. Block of I_{Na} prevents pacing-induced arrhythmias

(A) Effect of treatment with β -adrenergic receptor blocker (BB) propranolol on heart rate in WT and *Mecp2*^{Null/Y} mice. (B) Effect of propranolol on QTc intervals in WT and *Mecp2*^{Null/Y} mice. (C) Effect of propranolol on PES-induced arrhythmias in WT and *Mecp2*^{Null/Y} mice. Two *Mecp2*^{Null/Y} mice developed severe atrioventricular block (AVB) and the other three developed sustained VT or ventricular fibrillation (VT/VF). (D and E) Effect of PHT on late phase I_{Na} in *Mecp2*^{Null/Y} cardiomyocytes, WT control cardiomyocytes, and NKO cardiomyocytes. (F) Effect of acute PHT injection on heart rate in WT and *Mecp2*^{Null/Y} mice. (G) Effect of PHT on QTc interval in WT and *Mecp2*^{Null/Y} mice. (H) Effect of PHT on arrhythmias in *Mecp2*^{Null/Y} and WT mice. * $P < 0.05$, ** $P < 0.01$. N is indicated in bar graphs. NS, non-significant. pA=pico-Amperes, pA/s = pA per second, the integrated magnitude of the current from 350–800ms after depolarizing pulse.

Table 1
Electrophysiological intervals in WT and *Mecp2*^{Null/Y} mice at 2–3 months old

Data are expressed as mean \pm SEM. RR, time interval between two consecutive RR waves; PQ, interval from the beginning of the P wave to the peak of the Q wave; QRS, duration of the interval between beginning of Q wave to peak of S wave; QT, interval from beginning of Q wave to the end of the T wave; QTc, QT interval corrected for heart rate; SCL, sinus cycle length time; AV, interval from the beginning of the P wave to the beginning of the QRS complex.

	WT (n = 11)	<i>Mecp2</i> ^{Null/Y} (n = 12)
RR (ms)	118.3 \pm 4.6	113.8 \pm 3.0
PQ (ms)	35.3 \pm 1.5	35.3 \pm 1.6
QRS (ms)	8.9 \pm 0.3	10.6 \pm 0.4 **
QT (ms)	37.3 \pm 1.7	49.8 \pm 2.0 **
QTc (ms)	53.7 \pm 2.2	67.6 \pm 1.8 ***
SCL (ms)	114.2 \pm 3.4	117.9 \pm 3.4
AV (ms)	37.5 \pm 1.5	39.5 \pm 1.9

**
 $P < 0.01$,

 $P < 0.001$ versus WT.

Table 2
Echocardiographic parameters of WT and *Mecp2^{Null/Y}* mice at 2–3 months of age

Data are expressed as mean \pm SEM. HR, heart rate; bpm, beats per minute; EF, ejection fraction; FS, left ventricular fractional shortening; ESD, end-systolic diameter; EDD, end-diastolic diameter; IVSs/IVSd, intraventricular septal wall thickness in systole/diastole; LVPWs/LVPWd, left ventricular posterior wall thickness in systole/diastole.

Age	3 months	
	WT (n = 8)	<i>Mecp2^{Null/Y}</i> (n = 8)
HR (bpm)	478.2 \pm 39.7	536.4 \pm 57.1 *
EF (%)	65.2 \pm 6.2	70.7 \pm 5.9
FS (%)	35.3 \pm 4.6	39.5 \pm 4.8
ESD (mm)	2.5 \pm 0.4	2.1 \pm 0.3
EDD (mm)	3.8 \pm 0.3	3.5 \pm 0.2
IVSs (mm)	0.96 \pm 0.06	0.95 \pm 0.08
IVSd (mm)	0.7 \pm 0.1	0.8 \pm 0.1
LVPWs (mm)	1.09 \pm 0.15	1.18 \pm 0.09
LVPWd (mm)	0.8 \pm 0.1	0.8 \pm 0.1

* $P < 0.05$ versus WT.

Table 3
Electrophysiological intervals recorded in WT and *Mecp2*^{+/-} mice

Data are expressed as mean \pm SEM. RR, time interval between two consecutive RR waves; PQ, interval from the beginning of the P wave to the peak of the Q wave; QRS, duration of the interval between beginning of Q wave to peak of S wave; QT, interval from beginning of Q wave to the end of the T wave; QTc, QT interval corrected for heart rate; SCL, sinus cycle length time; AV, interval from the beginning of the P wave to the beginning of the QRS complex.

	WT 4 m.o. (n = 5)	<i>Mecp2</i> ^{+/-} 4 months (n = 13)	WT 10 months (n = 6)	<i>Mecp2</i> ^{+/-} 10 months (n = 7)
RR (ms)	122.4 \pm 4.7	121.7 \pm 5.2	117.7 \pm 2.1	116.9 \pm 3.1
PQ (ms)	31.6 \pm 2.7	33.5 \pm 3.2	30.0 \pm 0.8	31.3 \pm 0.8
QRS (ms)	8.2 \pm 0.5	8.9 \pm 0.9	7.5 \pm 0.3	8.6 \pm 0.3 [*]
QT (ms)	35.6 \pm 1.5	35.8 \pm 2.2	33.7 \pm 1.0	42.3 \pm 1.4 ^{***}
QTc (ms)	50.7 \pm 1.6	51.2 \pm 1.9	50.3 \pm 0.6	58.1 \pm 1.5 ^{***}
SCL (ms)	122.6 \pm 4.7	122.5 \pm 5.2	117.7 \pm 2.2	116.9 \pm 3.1
AV (ms)	38.8 \pm 3.0	39.8 \pm 3.2	37.3 \pm 1.6	39.1 \pm 1.7

^{*} $P < 0.05$,

^{***} $P < 0.001$ versus age-matched WT.

Table 4
Echocardiographic parameters of WT and *Mecp2*^{+/-} mice

Data are expressed as mean \pm SEM. HR, heart rate; bpm, beats per minute; EF, ejection fraction; FS, left ventricular fractional shortening; ESD, end-systolic diameter; EDD, end-diastolic diameter; IVSs/IVSd, intraventricular septal wall thickness in systole/diastole; LVPWs/LVPWd, left ventricular posterior wall thickness in systole/diastole.

Age	4 months		12 months	
	WT (n = 9)	<i>Mecp2</i> ^{+/-} (n = 8)	WT (n = 7)	<i>Mecp2</i> ^{+/-} (n = 8)
HR (bpm)	380.0 \pm 7.1	392.4 \pm 11.3	388.1 \pm 11.5	402.5 \pm 8.2
EF (%)	66.5 \pm 2.0	66.5 \pm 1.2	63.6 \pm 2.5	67.5 \pm 0.9
FS (%)	36.3 \pm 1.4	36.2 \pm 0.9	34.4 \pm 1.9	37.2 \pm 0.7
ESD (mm)	2.45 \pm 0.10	2.40 \pm 0.07	2.70 \pm 0.14	2.69 \pm 0.10
EDD (mm)	3.84 \pm 0.09	3.77 \pm 0.05	4.10 \pm 0.12	4.24 \pm 0.12
IVSs (mm)	0.98 \pm 0.01	0.98 \pm 0.01	0.99 \pm 0.01	1.06 \pm 0.02 *
IVSd (mm)	0.63 \pm 0.01	0.64 \pm 0.01	0.71 \pm 0.02	0.78 \pm 0.02 *
LVPWs (mm)	1.00 \pm 0.02	1.00 \pm 0.01	1.06 \pm 0.03	1.16 \pm 0.02
LVPWd (mm)	0.63 \pm 0.01	0.64 \pm 0.01	0.71 \pm 0.03	0.76 \pm 0.03

* $P < 0.05$ versus WT.