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Heart rate dynamics in monoamine oxidase-A- and -B-deficient mice

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Abstract

Heart rate (HR) dynamics were investigated in mice deficient in monoamine oxidase A and B, whose phenotype includes elevated tissue levels of norepinephrine, serotonin, dopamine, and phenylethylamine. In their home cages, spectral analysis of R-R intervals revealed more pronounced fluctuations at all frequencies in the mutants compared with wild-type controls, with a particular enhancement at 1–4 Hz. No significant genotypic differences in HR variability (HRV) or entropies calculated from Poincaré plots of the R-R intervals were noted. During exposure to the stress of a novel environment, HR increased and HRV decreased in both genotypes. However, mutants, unlike controls, demonstrated a rapid return to baseline HR during the 10-min exposure. Such modulation may result from an enhanced vagal tone, as suggested by the observation that mutants responded to cholinergic blockade with a decrease in HRV and a prolonged tachycardia greater than controls. Monoamine oxidase-deficient mice may represent a useful experimental model for studying compensatory mechanisms responsible for changes in HR dynamics in chronic states of high sympathetic tone.

Keywords

serotonin; norepinephrine; cholinergic; arrhythmia; heart rate variability; vagus nerve; sympathetic; parasympathetic

ENZYMATIC DEGRADATION by monoamine oxidase (MAO)-A and -B plays a crucial role in the regulation of tissue levels of serotonin (5-HT), norepinephrine (NE), epinephrine,

phenylethylamine, and dopamine. Our laboratory has recently identified (29) a natural mutation of MAO-A occurring in mice with targeted deletion of the MAO-B gene (14). These mice doubly deficient in both MAO isoforms [MAO-A/B knockout (KO)] demonstrate brain levels of 5-HT, NE, dopamine, and phenylethylamine that are increased 8.5-, 2.2-, 1.7-, and 15.7-fold above those noted in adult, wild-type (WT) animals, with levels of the 5-HT metabolite 5-hydroxyindoleacetic acid essentially undetectable in both the brain and urine.

When these neuroactive compounds are administered acutely, they show cardiac electrophysiological effects, including changes in the dynamics of the sinoatrial node and in the atrioventricular conduction, as well as in the ventricular fibrillation threshold (11, 18, 27, 31, 36). On the other hand, the results of chronic catecholamine elevations observed in patients are more mixed, showing both paroxysmal tachycardia as well as occasional bradyarrhythmias (3, 28, 39). The elevated bioamine levels observed in the MAO-A/B KO mice since early development could certainly alter their cardiovascular autonomic control; however, it is not immediately apparent how the entire system may (or may not) adapt during the animal's lifespan. The purpose of this study was to characterize the cardiac response to this unique amine overload as reflected in heart rate (HR) dynamics.

METHODS

Animals

The subjects reported in this study were adult, male MAOA/B-deficient mice (age = 20.9 ± 0.8 wk, 24.3 ± 0.5 g body wt, $n = 8$, means $\pm$ SE) (29) and their WT counterparts (age = 25.4 ± 1.1 wk, 30.5 ± 0.7 g body wt, $n = 11$). All procedures performed were reviewed and approved by the Animal Care and Use Committee at the University of California at Los Angeles. The background strain of the animals was that of MAO-B KO mice (14), originally generated in a C57-BL6/129Sv strain, whose males were subsequently backcrossed over 10 generations with 129Sv females. Breeding of these animals has revealed an X-linked inheritance with affected male offspring demonstrating absence of both MAO-A as well as MAO-B enzymatic activity. Absence of MAO-A mRNA and MAO-A protein in these animals has been confirmed, respectively, by Northern and Western blot analyses, although the defect in MAO-A at the gene level has yet to be determined (29). At the end of the experiment, absence of the MAO-B gene was confirmed in MAO-A/B KO mice by a polymerase chain reaction of DNA prepared from tails (6) (data not shown) as well as by measurement of MAO-A enzymatic activity in the liver (MAO-A/B KO: 0.077 ± 0.021 nmol·20 min⁻¹·mg protein⁻¹, $n = 8$; WT: 5.52 ± 0.69 nmol·20 min⁻¹·mg protein⁻¹, $n = 10$) using methods previously reported (15).

Surgical Placement of the Telemetry Implants

Mice were anesthetized with halothane (2.0% induction, 1.2% maintenance) in 30% oxygen and 70% nitrous oxide. Rectal temperature was maintained at 36.5°C with a BAT-12 thermocouple thermometer connected to a TCAT-1A temperature controller (Physitemp; Clifton, NJ), a heating pad, and a source of radiant heat. Implantation of a radiotransmitter (model TA10ETA-F20, Datasciences; St. Paul, MN) was done using methods previously

reported (21). With the use of aseptic techniques, a 2-cm incision was made along the abdominal midline immediately caudal to the xyphoid process. The radiotransmitter was inserted into the abdominal cavity and secured via four sutures to the parietal layer of the peritoneum and the rectus abdominus fascia. Two leads were tunneled through the subcutaneous space to reach the thoracic wall overlying the apex of the heart and the right acromion, where they were secured with one stitch. The rectus abdominus fascia and overlying skin were sutured, and the animal was allowed to recover. Animals were housed singly in Plexiglas cages with contact bedding and ad libitum food and water. A 24-h diurnal light cycle was maintained, with lights on from 07:00 to 19:00 (day) and lights off from 19:00 to 07:00 (night). Implants could be turned on or off with an external magnet, sending a radiofrequency signal to a receiver platform placed underneath the animal's home cage. The receiver platforms were linked via a data exchange matrix to a microcomputer in an adjacent, dedicated vivarium room. Dataquest ART 2.0 Data Acquisition software (Datasciences) was used for digitization of the signal (at a sampling rate of 1,000 Hz) and for on-line display of the electrocardiogram (ECG) as well as for data storage onto a hard disk.

Recording Protocols

All recordings were begun 2 wk after the placement of the telemetry implant, consistent with previous work suggesting a minimum of 4 days for resumption of normal circadian parameters (8). Recordings were performed within our own designated vivarium suite at ambient room temperatures of 22.2–24.9°C. Results in this paper are from data collected using the following recording protocols.

Unperturbed recordings in the home cage—The first group of animals was studied for 36 consecutive hours. A segment of 5 min of continuous ECG was collected each hour.

Novel environment—The same subjects were exposed to a novel environment to investigate possible effects on HR and rhythm. In this paradigm, animals were removed from their home cage and placed into an unfamiliar clear Plexiglas cage (rectangular, 40 × 20 × 16 cm, without bedding, placed under attenuated light). An ECG was recorded during 10 min before, while animals were still in their home cage, as well as during 10 min of residence in the novel environment.

Vagal blockade—Effects of cholinergic blockade were examined in MAO-A/B KO mice and WT mice in their home cages. Twenty minutes of continuous baseline ECG were recorded, after which time the animals were briefly removed and administered 0.9% saline (0.2 ml ip), after which they were returned to their home cages for an additional 60 min of recording. The following day this protocol was repeated, substituting the saline injection for glycopyrrolate (0.1 mg/kg ip), a muscarinic blocker devoid of central effects.

Data Analysis

Stored signals were processed using programs written in Matlab (MathWorks; Natick, MA). These routines were used to calculate R-R intervals from the ECG records. Briefly, this involves first selecting a representative QRS complex as a template. The algorithm then

scans the record, calculating the cross-correlation between the template and the time series. In a second pass, the algorithm identifies the points in the time series with maximum correlation, labeling those as R waves.

Temporal statistics—HR variability (HRV) in the time domain was analyzed according to the methods recommended by the task force of HRV in humans (35). For each mouse in its unperturbed home cage environment, mean R-R intervals and standard deviations of the R-R intervals (SD R-R) were calculated over 5 min of recording for MAO-A/B KO mice ($n = 8$) and WT mice ($n = 11$). More extended data sampling in four MAO-A/B KO and three WT mice was also undertaken. In these recordings, 5-min windows recorded every hour during 36 consecutive hours were analyzed. The analysis was separated into three night/day/night segments, each of 12-h duration. Mean R-R intervals and SD R-R were then averaged for each mouse across each 12-h period, and group averages ($\pm$ SE) were calculated across genotypes.

During the exposure to the novel environment, R-R intervals were averaged for each animal across each 20-s time interval, and group means $\pm$ SD were determined. During the pharmacological challenge, R-R intervals for each animal were averaged over a 20-min continuous baseline before drug administration; a second average was calculated across 60 min of continuous recording after drug administration. Group means $\pm$ SD were compared using a *t*-test (2-tailed, $P < 0.05$).

Statistical comparison of genotypic differences was performed with a univariate repeated-measures ANOVA, with the specific details described for each protocol in RESULTS.

Frequency measures—The spectral domain analysis of the tachogram (35) was done on a frequency range commonly used in rodents (0.03–4 Hz) and was based on ECG recordings of 5-min duration recorded every hour over 50 continuous hours. In brief, the spectral power of the R-R interval was determined from the resampled tachogram using a fast Fourier transform algorithm. To obtain an equidistant time series, the tachogram was resampled by linear interpolation at 40.96 Hz. Spectral power was calculated on 50% overlapped blocks of 4,096 points (i.e., 100 s). Linear trend removal was applied to the time series before calculation of the power to prevent high-frequency (HF) artifacts.

Beat-to-beat dynamics—Fast fluctuations of the R-R intervals were examined using Poincaré plots. This plot is a graph in which consecutive R-R interval pairs are plotted with the n th R-R period plotted in the ordinate against the n th+1 R-R period in the abscissa. This graph can be examined, revealing qualitative information about rate-dependent variability. They were quantitatively analyzed as well by calculation of the entropy of the distribution of the R-R interval pairs. This estimates the degree of the two-dimensional spread of the R-R pairs. Thus this measure equals zero for perfect order, for example, in the case of a perfectly steady pacemaker with identical R-R intervals, and is maximum in the extreme of a completely random sequence of intervals. The technique (25) involves computation of the equation ****where $H(i, j)$ is the entropy, $\log$ is the logarithm on base 2, and $P(i, j)$ represents the probability of finding an R-R interval of length i followed by an R-R interval of length j . The sum is done over the normalized probabilities in each and all cells in the

array. It is necessary to choose a given partition of the R-R intervals and a maximum range for the distribution, for which we used 1 and 256 ms, respectively. These values determined the maximum entropy to be 16 bits.

RESULTS

Short-Term HR Dynamics

A typical ECG record can be examined in Fig. 1. Data in this figure were gathered from a MAO-A/B KO mouse in its home cage. Figure 1, *middle*, shows 8 s of ECG, and Fig. 1, *bottom*, shows the corresponding R-R interval time series. Of note are the abrupt changes in HR of sinoatrial node origin, as evidenced by the similarity of QRS and P wave latency and morphology plotted in Fig. 1, *top*. We did not observe, despite careful examination, instances of ectopy in either strain.

Temporal Statistics of Unperturbed Home Cage Recordings

Mean R-R intervals and SD were calculated in 5-min windows recorded every hour during 36 consecutive hours. The analysis was separated into three night/day/night segments, each of 12-h duration, resulting in the following group means: WT ($n = 3$), first night 107.3 ± 3.2 ms, first day 118.3 ± 2.2 ms, second night 110.7 ± 3.8 ms; and MAO-A/B KO ($n = 4$), first night 109.5 ± 11.6 ms, first day 111.8 ± 6.4 ms, second night 109.2 ± 4.0 ms (means $\pm$ SE). There were no significant genotypic differences ($P > 0.2$).

The group SD of the R-R intervals for the same night/day/night segments were as follows: WT ($n = 3$), 13.8 ± 2.8 , 12.7 ± 2.6 , and 13.0 ± 1.7 ms; and MAO-A/B KO ($n = 4$), 17.7 ± 5.5 , 18.3 ± 4.9 , and 18.7 ± 5.9 ms (mean SD R-R $\pm$ SE). There were no significant genotypic differences ($P > 0.2$). This was confirmed when mean R-R intervals and SD R-R were calculated over 5-min recordings in a larger sample of animals [MAO-A/B KO ($n = 8$): mean R-R = 97.6 ± 11.4 , SD R-R = 5.8 ± 3.0 ; WT ($n = 11$): mean R-R = 104.1 ± 12.1 , SD R-R = 6.1 ± 2.3 ; P value for mean R-R = 0.25, P value for SD R-R = 0.84].

Beat-to-Beat Dynamics

The beat-to-beat dynamics of these fluctuations were examined using Poincaré plots (Fig. 2). For each animal, consecutive pairs of R-R intervals were graphed with the n th R-R interval (x -axis) plotted against the n th+1 R-R period (y -axis). Differences in interbeat variability were quantified by measuring the entropy of the distribution of points in the Poincaré plots. In these plots, increasing R-R intervals resulted in an increase in variability in both genotypes, as has been similarly reported in other species (35). In the analysis of the whole dataset of the three 12-h night/day/night segments, the calculated entropies for WT ($n = 3$) mice were 8.03 ± 0.38 , 8.06 ± 0.63 , and 7.98 ± 0.36 bits on a logarithmic scale and for MAO-A/B KO ($n = 4$) mice were 8.34 ± 1.33 , 8.55 ± 0.71 , 8.42 ± 0.77 bits on a logarithmic scale (means $\pm$ SE). Entropies of mutant animals in their home cages did not differ significantly from controls ($P > 0.2$). This was confirmed when entropies were calculated over 5-min recordings in a larger sample of animals [MAO-A/B KO ($n = 8$): 6.84 ± 1.36 ; WT ($n = 11$): 7.51 ± 0.83 , $P > 0.2$].

Frequency-Domain Analysis of Home Cage Recordings

MAO-A/B KO mice demonstrated a greater power in all frequencies of the HF region of the power spectrum (frequency domain). After the recordings were processed and the R-R intervals were extracted, the Fourier analysis resulted in the plots of Fig. 3. In Fig. 3, the thick and thin lines represent group averages of power spectra for MAO-A/B KO ($n = 4$) and WT mice ($n = 3$), respectively, obtained during the day (A) and night (B). Note the inverse law between power and frequency, which is typical of HRV data (i.e., lower frequencies exhibit larger fluctuations). In addition, in the MAO-A/B KO mice, there was a pronounced increase in power in the 1- to 4-Hz band. For the three frequency ranges, these differences were close to an order of magnitude, which were statistically significant ($P < 0.01$).

HRV During a Behavioral Stressor

The recording carried out during exposure of the animals to a novel environment reveals a different time course of HR changes for WT mice (sustained tachycardia throughout the observation period) than for MAO-A/B KO mice (initial tachycardia with subsequent return to baseline) (Fig. 4A). When data obtained over the entire baseline period spent in the home cage were considered, repeated-measures ANOVA revealed no significant effect of the factor genotype on mean R-R interval [MAO-A/B KO ($n = 8$) mean R-R: 113.1 ms; WT ($n = 11$) mean R-R: 116.4 ms], but a significant effect of genotype on SD R-R, a time-domain indication of the enhanced HRV of MAO-A/B KO mice (MAO-A/B KO: 19.2 ms; WT = 9.9 ms, $P < 0.0001$). When the same analysis was performed with animals in the novel environment, the mean R-R interval was longer in the MAO-A/B KO mice (116.4 ms) than in the WT mice (86.6 ms, $P < 0.01$). During this behavioral activation, SD R-R did not significantly differ between MAO-A/B KO (12.1 ms) and WT mice (13.8 ms). During behavioral activation, there was no significant genotypic difference in locomotor activity ($P < 0.1$; Fig. 4B). Although locomotor activity tended to be lower in the mutant animals, differences in the R-R interval persisted even at times when activity counts were similar.

Figure 5 demonstrates the dependence of the SD on the averaged R-R intervals, as recorded in mice of both genotypes in their home cages. Data points for the MAO-A/B KO mice obtained for the home cages clustered around a common regression line (slope = 0.48, y-intercept = -36.4, F value of regression = 117.2, $P < 0.00001$). This behavior persisted despite shortening of the R-R interval when the animals were moved to a novel environment. In contrast, a similar plot from WT mice did not demonstrate a dependence of SD R-R on R-R interval, and data from home cages and the novel environment appear as two distinct clusters with no continuity between them for this genotype. A similar relationship was found between R-R mean and the root mean square of successive differences (data not shown).

HRV During Vagal Blockade

Saline injection resulted in an immediate decrease in the R-R interval (Fig. 6C). This change was similar in WT ($n = 8$) and MAO-A/B KO mice ($n = 4$) and recovered progressively during the course of the observation period. No change in HRV was observed between the genotypes as evidenced by similar SD (Figs. 6D and 7B). This transient acceleration of HR was interpreted to be a consequence of stress associated with manipulation of the animals

and the trauma of injection. Glycopyrrolate administration was followed by a reduction of the R-R interval duration in both strains (Fig. 7A), greater than that observed with saline ($P = 0.019$; Fig. 7B). In response to glycopyrrolate, R-R intervals in WT animals ($n = 8$) gradually increased to baseline over the duration of the observation period, whereas in MAO-A/B KO mice ($n = 4$) they remained low throughout, indicating a prolonged tachycardia (Fig. 6A). A reduction in HRV (SD R-R) in response to glycopyrrolate was observed only in MAOA/B KO mice (Figs. 6B and 7A).

DISCUSSION

Several relevant differences in HR dynamics were identified in this study between WT and MAO-A/B KO mice. First, in the unperturbed environment of their home cages, spectral analysis of the R-R intervals revealed more pronounced fluctuations at all frequencies in the mutant mice compared with controls, with a particular enhancement of modulation in the range of 1–4 Hz. Temporal statistics obtained in the home cage of the animals showed no significant genotypic difference in HRV or entropies calculated from the respective Poincaré plots. Second, during exposure to the stress of a novel environment, HR increased and HRV decreased in both genotypes. However, a different time course of HR changes was seen in WT mice (sustained tachycardia throughout the observation period) than in MAO-A/B KO mice (initial tachycardia with subsequent return to baseline). That such modulation may be the result of an enhanced vagal tone was suggested by observations that MAO-A/B KO mice responded to cholinergic blockade with a decrease in HRV, as well as a prolonged tachycardia, greater than that observed in WT mice.

The autonomic effects of chronic elevations of the catecholamines or indoleamines remain controversial. Because the genetic mutation in the MAO-A/B KO mice exists from the earliest stages of embryogenesis throughout development, phenotypes examined in adulthood will likely include adaptive changes that occur in response to the absence of the gene (16). The presence of physiological adaptation is increasingly being described in a number of genetic mutant mice, including reports of compensatory changes in the localization, density (30), and sensitivity (7) of postsynaptic receptors, and changes in cell metabolism (38). Reports have, however, usually focused on isomodal changes, that is, changes that occur within the same neurochemical system as that of the genomic ablation. For example, deletion of the MAO-A gene in MAO-A-deficient mice results in a significant isomodal increase in its preferred substrate 5-HT (6, 19) as well as a down-regulation of postsynaptic 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors (33). Compensatory changes, however, may also occur in separate neurochemical systems (cross-modal). Sympathetic and parasympathetic regulation of the cardiac rhythm are tightly coupled. The results of the current study suggest that MAO-A/B KO mice compensate their high adrenergic state by an enhanced vagal tone. During behavioral activation elicited by the animal's exploration of a novel environment, both MAO-A/B KO and WT mice demonstrated an initial increase in HR to near maximal levels, with a concomitant decrease in HRV. Over the course of the 10-min observation period, mutant mice displayed a more rapid return to baseline HR along with a marked increase in HRV. We hypothesized that such changes resulted from an exaggerated vagal recruitment whose effect was to decelerate cardiac rhythms in face of the elevated adrenergic tone. In a separate paradigm in which behavioral activation was elicited

by the stress of administration of an intraperitoneal injection, suppression of vagal activity with glycopyrrolate completely eliminated this compensatory effect in the mutant mice. In fact, HR remained high (short R-R intervals) and HRV remained low for the duration of 1 h after drug injection. In contrast, WT mice slowed their HR (increased R-R interval), probably by a decrease in adrenergic tone, a mechanism most likely compromised in MAO-A/B KO mice due to their inability to rapidly metabolize excessive tissue levels of catecholamines and indoleamines. Thus the sudden decelerations of HR observed in MAO-A/B KO mice (Fig. 1) were most likely due to enhanced vagal tone rather than to reduced adrenergic tone. This may be relevant to understanding the tachy- as well as bradyarrhythmias reported in patients with pheochromocytoma, in whom chronic elevations of catecholamines are associated with compensatory elevations in vagal tone (10, 24).

HR is well known to be dependent on locomotor activity. In our study, significant differences in the HR response were observed during exposure to the stress of a novel environment despite the absence of a significant genotypic difference in locomotor activity. Although locomotor activity tended to be lower in the mutant animals, differences in the R-R interval persisted even at times toward the end of the exposure, when activity counts were similar. Preliminary results from our laboratory suggest that differences in the cardiac response of MAO A/B KO mice seen in the current study are similar to that obtained when animals are immobilized in a Plexiglas tube maintained on a heating pad set at 36.5°C (personal communication). Such observations suggest that the animal's HR responses to stress are independent of differences in locomotor activity.

In so far as HR and body temperature are closely linked, an important aspect is the thermal response of the animals. A limitation of our current study is that we did not specifically measure temperature regulation or cardiac responses to such factors as cold-induced stress. We believe that possible differences in temperature regulation are not primary in determining the HR alterations observed in the current study. This is supported by the fact that genotypic differences obtained in the spectral analysis were seen both at night as well as during the day, suggesting that such differences are observable independent of the animal's circadian temperature regulation. Furthermore, recent telemetry recordings from our laboratory show that genotypic differences in HR are observed at times during the light phase, when MAO-A/B KO and WT mice show no difference in core temperature or locomotor activity (personal communication).

Changes in sinoatrial rhythm and vagal tone in the MAO-A/B KO mice might occur via several possible mechanisms. Such changes may reflect functional and/or structural abnormalities at the level of the central nervous system, the peripheral nervous system, the vasculature, or sinoatrial node. Increases in parasympathetic tone may be due directly to increased recruitment of existent cholinergic fibers or indirectly through activation of noncholinergic fibers that are pre- and postsynaptic to vagal terminals. It has been suggested, for instance, that activation of presynaptic 5-HT receptors may change neurotransmitter release from vagal afferent neurons and thus may modulate the cardiac reflex responses through mechanisms linked to alterations in vagal tone (17, 22, 32). Furthermore, it is well known that vagal slowing of the HR increases during noradrenergic stimulation. Thus, during infusion of NE characterized by accentuated sympathovagal

antagonism, HR becomes remarkably unstable, an observation attributed primarily to sudden vagal bursts or withdrawals (37). ACh and NE at the level of the sinoatrial node show different temporal influences, and it has been suggested that the faster cholinergic response may relate to the more direct G protein-mediated channel pathway of vagal effects as opposed to the slower response of the noradrenergic effects acting through a slower cAMP-protein kinase pathway (4).

Spectral analysis of HRV has been used in the past as a qualitative measure to assess sympathetic/parasympathetic balance. Whereas HF power is widely accepted as a marker of cardiac parasympathetic control, modulated by breathing, low-frequency (LF) power has contributions from both vagal and sympathetic inputs (1, 26, 34). Increased sympathetic activity is characterized by a shift in favor of the LF component, whereas during increases in vagal activity the HF component predominates (12, 20). These HF and LF components present in R-R intervals are highly coherent with muscle sympathetic nerve activity as well as systolic arterial pressure variabilities (12, 23). Alterations in the LF-to-HF ratio with pharmacological blockade suggests that the control of HRV is similar in mice and humans, with the mouse LF component (0.4–1.5 Hz) regulated by both sympathetic and vagal inputs and the mouse HF component (1.5–4 Hz) predominantly parasympathetically mediated (13). MAO-A/B KO mice compared with WT mice showed increased power at all spectral frequencies, with a particular enhancement of modulation in the range of 1–4 Hz, consistent with an overall increase in parasympathetic tone. The increases in the 1- to 4-Hz band are in the range of frequencies of murine respiration (9) and likely reflect an altered respiratory modulation of HRV. Diaphragmatic dysfunction may impair the breathing pattern and, hence, artificially alter respiration as a basic constituent of neurocardiovascular control (13). Recent research has shown that MAO-A-deficient mice (MAO-A KO) demonstrate abnormalities in morphology and activity of the phrenic motoneurons (2, 5). These abnormalities likely originate from an excess of 5-HT, because prenatal treatments inhibiting 5-HT synthesis or treatment with a 5-HT_{2A} receptor antagonist reinstitute normal phrenic motoneuron morphology and activity in MAO-A KO mice. Whether similar neuronal abnormalities can be observed in MAO-A/B-deficient mice remains to be clarified. Furthermore, spectral differences between MAO-AB KO and WT mice will require more extensive study to delineate, given the small number of animals used in our study.

In conclusion, this study shows that mice deficient in MAO-A and -B demonstrate altered HR dynamics during a behavioral challenge, whereas less pronounced genotypic differences were exhibited in the home cage environment. Pharmacological infusion studies suggest that this differential response might be related to a compensatory enhanced cholinergic tone. The uncovered differences between mutant mice and their WT counterparts may further our understanding of the several pathophysiological consequences of states with high sympathetic/parasympathetic tone.

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Fig. 1. Representative time series of electrocardiogram (ECG; *middle*) and corresponding R-R intervals (*bottom*) of a monoamine oxidase (MAO)-A/B knockout (KO) mouse. *Top*: fragment of the ECG record at a faster time base.

Fig. 2.

Poincaré plots in which 500 consecutive pairs of R-R intervals are graphed with the n th R-R interval (x -axis) plotted against the n th+1 R-R period (y -axis). Note that increasing heart rate (HR) results in a decrease in variability, seen as a tightening of the scatter along the diagonal line for short R-R intervals and vice versa. Depicted are examples of an animal with a high entropy (A) and a low entropy (B).

Fig. 3.

Frequency-domain analysis of HR variability (HRV) during the day (7:00–19:00) (A) and night (19:00–7:00) (B). Relative to wild-type (WT) mice ($n = 3$), the MAO-A/B KO mice ($n = 4$) exhibited more pronounced fluctuations at all frequencies, but also an enhanced modulation of the 1–4 Hz range. Denoted are the different frequency ranges: high (HF; 1–4 Hz), low (LF; 0.08–1 Hz), and a very-low-frequency range (VLF; 0.03–0.08 Hz). Each spectrum represents the average of 12 segments of 5-min duration each. Note that the axes are logarithmic.

Fig. 4.

Genotypic differences in the effect of a novel environment on R-R intervals. Depicted are the mean R-R $\pm$ SD (A) and mean locomotor activity $\pm$ SD (B) in MAO-A/B KO mice ($n = 8$) and WT mice ($n = 11$). The *left* series of measurements was taken while animals were unperturbed in their home cages, and the *right* series of measurements were taken immediately after they were placed in a novel environment (a clear Plexiglas cage). Each data point represents the average over 20 s.

Fig. 5.

Dependence of the SD of the R-R interval on mean R-R interval during exposure to a novel environment. The same data points as in Fig. 4 are replotted for MAO-A/B KO mice (A) and WT controls (B).

Fig. 6.

Prolonged tachycardia and increased HRV after cholinergic blockade in MAO-A/B KO mice. Depicted are R-R intervals before and after either intraperitoneal glycopyrrrolate (A) or intraperitoneal saline administration (C). The raw data of all R-R intervals of MAO-A/B KO mice ($n = 4$) and WT mice ($n = 8$) were acquired over a 20-min baseline, followed by injection (time = 0) and subsequent data acquisition over 60 min. The R-R interval frequency histograms for each postglycopyrrrolate (B) or postsaline period (D) are also shown.

Fig. 7.

Mean R-R intervals and SD before and after administration of either glycopyrrolate (A) or saline (B) (same data as in Fig. 6, A and C). Averages were computed over the entire 20-min baseline or over the entire 60 min after drug administration. Compared with baseline conditions, glycopyrrolate produced a significant ($*P = 0.019$) reduction in R-R intervals. All other differences were nonsignificant.