Articles in PresS. Physiol Genomics (March 29, 2005). doi:10.1152/physiolgenomics.00085.2004

The α7 nicotinic acetylcholine receptor subunit is not required for parasympathetic control of the heart in the mouse.

Running Title: α7 deficient mice display normal resting autonomic tone

Jennifer Deck [1]

Steve Bibevski [2]

[1] Dept. Veterans Affairs Medical Center
Cleveland, OH 44106

[2] Dept. Physiology & Biophysics
School of Medicine, Case Western Reserve University
Cleveland OH 44106

Tomaso Gnecchi-Ruscone [3]

Valentina Bellina [3]

Nicola Montano [3]

[3] DiSC (Dipartimento di Scienze Cliniche), Ospedale Sacco, Università degli Studi di Milano, Milano, Italy

&

Mark E. Dunlap [1,2]

Corresponding Author:
Steve Bibevski MD/PhD
VA Medical Center 151W
10701 East Blvd, Cleveland OH 44106
steven.bibevski@case.edu
Tel: (216) 791-3800 X5114
Fax: (216) 707-5939

Abstract

Nicotinic acetylcholine receptors are assembled from a pool of 9 α and 3 β subunits into functional pentamers in peripheral autonomic neurons. The contribution of different subunits to native, physiologically important nAChR for synaptic transmission in autonomic ganglia is unclear. Here we examined the importance of the α7 subunit for parasympathetic innervation of the heart. **Methods**. Normal (C57BL/6J), α7 deficient (Chrna7), and wildtype littermate mice were implanted with telemetry devices and under conscious, unsedated conditions, ECG recordings were obtained at baseline and following atropine, propranolol, and hexamethonium bromide administration. Spectral analysis of heart rate variability (PSA) was performed for evaluation of resting autonomic tone to the heart. At the completion of conscious studies, animals were anesthetized and underwent electrical stimulation of the vagus nerve (VS) while recording R-R intervals. **Results**. Heart rate at baseline, after atropine, propranolol or hexamethonium was similar in all three groups of animals. PSA curves were similar between normal, wildtype and Chrna7 mice. VS showed no difference between control and Chrna7 mice throughout the range of stimulation (5-20 Hz). **Discussion**. Mice deficient in the α7 nAChR subunit do not display differences in resting autonomic tone to the heart at baseline or under conditions of single and combined autonomic blockade. Electrical stimulation of the vagus nerve showed no difference in heart rate responses between normal and α-7 deficient mice. These data support previous findings in vitro and highlight the important differences in function between nicotinic receptor subtypes since α3 deficient mice display major autonomic dysfunction. We conclude, that the α7 subunit does not contribute critically to resting parasympathetic control of the heart.

Introduction.

Synaptic transmission across sympathetic and parasympathetic neuronal ganglia in mammalian species is mediated by nicotinic acetylcholine receptors (nAChR). nAChR have been divided into neuronal and non-neuronal subtypes, based initially on sensitivity to blockade by methonium salts. More recently, it has become evident that neuronal nAChRs are assembled *in vivo* from a combination of 9 different α, and 3 different β subunits that form a functional ligand gated ion channels in various stoichiometries. An exception to this rule relevant to mammalian species has been the α7 subunit which in the nervous system most likely forms a homomeric pentamer (6) although heteromeric pentamers containing α7 are possible (23). Currently, the exact subunit composition of nAChRs responsible for ganglionic transmission in autonomic neurons to the mammalian heart remains unknown. We have previously shown in the canine cardiac ganglion that nAChR containing α3/β2 subunits, are the primary native receptor species that mediate synaptic transmission in efferent pathways to the heart with a possible smaller role for α7 containing receptors (2). Others have shown that α7 deficient mice may have altered baroreceptor function in the sympathetic efferent limb as well as altered sensitivities to hexamethonium, despite normal parasympathetic function (7). Previous work has shown that α7 subunit containing pentamers (which are sensitive to α-bungarotoxin)(1; 24) contribute a significant component of the excitatory post-synaptic current (EPSC) in chick ciliary ganglia (22), as well as rat (4), mouse (18) and chick sympathetic neurons (23). However, pharmacologic blockade of α7 containing receptors with α-bungarotoxin does not block synaptic transmission, indicating the importance of other subunits in mediating ganglionic transmission. In addition, the localization of the α7 subunit has

been shown to be at the periphery of the synaptic end-plate and not in the main functional domain (21) (8) which has added to the ambiguous role of α7 containing receptors. A possible functional role for the α7 subunit was suggested by Berg who showed that the α7 subunit was required for reliable synaptic transmission at higher stimulation frequencies (3). A role in ganglionic signaling for α7 containing receptors in cardiac neurons has been suggested by the presence of α7 mediated currents in rat intracardiac ganglia (4). These findings have indicated a possible role for α7 nAChR under artificial, *in vitro* conditions, but have failed to demonstrate a putative role of these receptors in normal physiology. Here, we examined the role of the α7 subunit to autonomic function in both the resting physiologic state as well as direct nerve stimulation studies *in vivo* in normal and α7 deficient mice.

METHODS.

Telemetry Implantation

All experiments were performed in accordance with guidelines for the care and use of research animals at Case Western Reserve University and the Cleveland Veterans Affairs Medical Center. 10 week old, 25-30g, male, C57BL/6J and Chrna7 mice were purchased from Jackson Labs, (Bar Harbor, ME). The α7-deficient mice were generated as described by Orr-Utregger (18). Two control groups were used. Wild type (C57BL/6-129s) + / + littermates of the Chrna7s, and regular C57BL/6J mice were studied to help detect any physiological differences between normal, and α7 deficient mice. The comparison between wildtype and regular C57BL/6J mice was done to determine if

regular C57BL/6J could be used in exchange for wildtype littermates given the restricted availability of the wildtype littermates.

All animals were anesthetized with 0.1ml per 20g body weight (IP) of a rodent cocktail anesthetic composed of 150mg Ketamine (100 mg/ml), 30mg Xylazine (20 mg/ml) and 5mg Acepromazine (10 mg/ml). Physiotel®, TA10EA-F20 telemetry devices (Data Sciences, St. Paul, MN), which were used to capture ECG signals in conscious, unsedated, freely-moving mice, were placed into the peritoneal cavity under sterile conditions without disruption to the viscera, and sutured with non-absorbable suture to the peritoneal wall. Leads were tunneled subcutaneously, and anchored to muscle in a lead II configuration. For the duration of the implant procedure, body temperature was maintained at 37˚C via Deltaphase Isothermal pads (Baintree, MA) and heart rate was monitored via ECG electrodes placed under the skin. 0.25% Marcaine (0.01mg/kg) was injected at incision sites to provide post-surgical analgesia. Following surgery, animals were housed individually in plastic cages fitted with sterile nesting material, allowed free access to tap water and fed standard mouse chow.

Recordings in Conscious Animals

All experimental recordings were performed weekdays during the hours of 8 a.m. and 3 p.m. Animals were not used for recordings on adjacent days and were selected for recordings randomly. Following adequate recovery from the surgical procedure (2 weeks), mice were placed into a round (15cm diameter) plethysmograph chamber containing bedding from their own cage 5 hours daily for an additional week in

order to acclimatize them to the new environment. ECG signals were recorded from within the plethysmograph chamber for two primary reasons. First, the chamber was large enough so that the animal was able to move freely without restraint, and second, it provided a controlled means by which the animal's respiratory pattern could be recorded. Prior to recordings, mice were permitted to acclimatize to the chamber until resting comfortably (approximately 1 hour). 5-minute baseline recordings were collected, followed thereafter by administration of either atropine sulfate (.04 mg/kg), propranolol (1 mg/kg) or hexamethonium bromide (25 mg/kg) intraperitoneally (IP). The animal was given 10 minutes to settle down following injection, and a 5-minute recording was repeated.

Protocol: Animals underwent single and combined autonomic blockade with three different experimental drug administrations selected randomly (see figure 1). All dosages were based on reported amounts in the literature where total blockade of the respective division was required and was confirmed in pilot studies in our lab before undertaking the protocol. Once a successful recording was collected, the next drug in the protocol was administered to the animal. In the case where hexamethonium was selected first, this was the only drug given on that day. The time from which the first drug was given was carefully monitored since recordings under subsequent drugs needed to be obtained during the active half-life of the previous drug. If there were issues that prevented obtaining recordings within the appropriate time frame, the experiment was discontinued and repeated at another date. Double-blockade experiments were usually completed within 30 minutes for atropine and 45 minutes for propranolol when given as first drugs

respectively. These time limits were well short of expected and tested washout of drug effectiveness.

Data Capture and Analysis

ECG signals were captured at 10KHz and stored on a personal computer using Ponemah, digital data acquisition and analysis system (Gould Instruments, Valley View, OH). Respiratory signals were obtained with a differential pressure transducer connected on one side to the recording chamber, and the other side to a sealed reference chamber. The chamber was custom-built from plexiglass (dimensions =15cm diameter X 12cm high) and was attached to a regulated vacuum source adjusted to provide continuous air flow at 1L/min. The recording chamber rested on a PhysioTel telemetry receiver (Data Sciences) for detection of transmitted ECG signal. All recordings were saved to hard drive and later replayed and inspected to ensure appropriate detection of ECG and respiratory signals. Assessment of resting autonomic tone to the heart in both control and the Chrna7 mice was done with spectral analysis of heart rate variability using autoregressive algorithms. The theoretical and analytical procedures have been described by us and others (5; 14; 20) . An automated routine generated a time series (tachogram) from the ECG signal. The respiratory signal was sampled once per cardiac cycle in order to obtain a time series synchronous with the tachogram. On stationary segments of RR interval time series (250-500 beats), autoregressive parameters were estimated via Levinson-Durbin recursion, and the order of the model was chosen according to Akaike's criterion (14; 17; 20). An autoregressive spectral decomposition was then performed. This procedure permitted us to automatically quantify the center frequency and the power of each

relevant component in absolute, as well as in normalized units (nu). The normalization procedure was performed by dividing the power of the low frequency (LF) or the high frequency (HF) component by the total spectral power from which the power of very low frequency (VLF) component had been subtracted, and multiplying the result by 100 (5; 14; 20). As previously reported (10), we selected <0.15 Hz, 0.15-1.5 Hz and 1.5-5 Hz as frequency ranges for VLF, LF and HF oscillations in mice (respectively). The monitoring of respiratory activity in our experiments allowed us to carefully evaluate the correspondence between respiratory rate and the HF component of R-R variability.

Vagus nerve stimulation protocol.

Animals were anesthetized with a rodent cocktail solution at a dosage of 0.1ml per 20g body weight (IP). This cocktail was composed of 150mg Ketamine (100 mg/ml), 30mg Xylazine (20 mg/ml) and 5mg Acepromazine (10 mg/ml). A tracheotomy was performed and the animal was ventilated using a MiniVent (Hugo Sachs Electronik) at a stroke volume of 300μL and at 250 breaths/minute. ECG signals were monitored via subcutaneous electrodes. The right vagus nerve was dissected and isolated at the cervical level through a single midline incision. The nerve trunks were ligated and sectioned at the cranial end and soaked with mineral oil. The caudal remnant of the nerve was then laid on the exposed ends of teflon-coated stainless steel wire electrodes with bared ends for contact to the nerve. Three sets of vagus nerve stimulations were performed at 5, 10, 15 and 20 Hz at 1mA and a pulse width of 1ms (Grass SD9 Stimulator). ECG signals were recorded for 15 seconds of baseline, during 10 seconds of continuous electrical stimulation and 15 seconds of recovery for each stimulation level performed. Prior to

subsequent stimulations, ample time was given to allow heart rate to return to resting levels. The vagal response was taken from the average R-R period during the entire stimulation period, which was instantaneous, and stable for the duration of stimulation.

Results.

Heart rate changes with pharmacologic blockade.

Resting heart rate (HR) was similar in control, wild-type and Chrna7 animals under baseline conditions, following atropine, propranolol, and after hexamethonium when each drug was given as first drug only (single blockade), (see Table 1) all $p>0.05$ by t-test. The change in heart rate (HR) in response to autonomic blockade using atropine (A), propranolol (B) and hexamethonium (C), is shown in Figure 2. Atropine caused a significant increase in HR in all three groups confirming prominent parasympathetic tone at rest in all three groups (figure 2A). Hexamethonium resulted in similar changes in HR compared to atropine suggesting that like most other mammals studied to-date, parasympathetic tone predominates at rest and the resting heart rate is determined primarily by vagal modulation (figure 2C). There was an expected bradycardia in response to propranolol in the C57BL/6J control group (decreased by 27 beats), which was not observed in the wildtype (increased by 8 beats) or Chrna7 group (increased by 5 beats), but the differences were not statistically significant ($p>0.05$) across all groups (figure 2B).

Table 2 shows power spectral analysis data for C57BL/6J control, wildtype and Chrna7 groups. HF and LF normalized units (nu) were similar across all three groups ($p>0.05$, t-

test) although the wildtype group had a large value in the LF component. This high value in the LF domain did not reach statistical significance compared to the other groups due to high inter-individual variability within the wildtype group. Figure 3 shows a representative power spectrum (PSD) from recordings obtained in mice under baseline conditions from each group. Animals displayed a significant HF component that correlated tightly to the respiratory frequency indicative of significant vagal modulation at rest. A LF component was also identified in each group at a similar frequency and power. Notably, the respiratory frequency was the same in each group of animals. Under conditions of autonomic blockade, the variance of R-R interval was abolished with atropine and hexamethonium, and dramatically reduced with propranolol, *Atropine*: Control = 0.7±0.3, wildtype 1.3±0.6, Chrna7 2.0±1.7 for reductions from baseline of 98.8, 99, and 97.6% respectively ($p>0.05$); *Propranolol* = Control 9.6±3.9, wildtype 1.7±0.7, Chrna7 30.2±19.3 for reductions from baseline of 79, 94 and 42% respectively ($p>0.05$); *Hexamethonium* = Control 3.5±2.8, wildtype 0.9±0.4, Chrna7 0.7±0.2 for reductions from baseline of 97, 99.7 and 99.3% respectively ($p>0.05$). Under this low variance state, specific frequencies could not be reliably detected and power spectral analysis following autonomic blockade was not performed.

Cumulative drug data obtained by sequentially adding the next drug in the protocol during the effective period of the preceding drug is shown in figures 4A & B. When propranolol was given as the second drug following atropine, there was a prolongation in R-R interval as expected. When propranolol was given as the first drug however, there was only a very small effect on R-R interval ($p>0.05$) suggesting that vagal tone

predominates at rest, and that sympathetic activity to the SA node is more apparent after blockade of vagal activity.

As seen in figure 1, hexamethonium was given following the administration of either atropine or propranolol in two different sequences. When hexamethonium was administered following propranolol as the second drug (figure 4A) there was an additional bradycardia of approximately 20-30 beats per minute ($p<0.05$), which suggested that a small degree of sympathetic tone remained. This may indicate a degree of incomplete blockade with the doses or atropine or propranolol used or a degree of washout of the previously administered drug. In comparison, when hexamethonium was administered following atropine (figure 4B), with propranolol as the first drug in the sequence, a smaller degree of bradycardia was observed ($p<0.05$). This suggested that the effect was not related to washout since there should have been more washout when propranolol was given earlier in the protocol. Since the doses of propranolol used were adequate for blockade of sympathetic activity, it is possible this small bradycardia resulted from decreased co-transmitter release from post ganglionic nerve terminals after hexamethonium.

Electrical Vagal Stimulation.

Vagal stimulation was done in order to elicit differences between the groups in a controlled, dose-response manner across a wide spectrum of intensities. Vagal stimulation resulted in an instantaneous onset of R-R prolongation, which was stable for the duration of stimulation. There was similar change in RR interval between the control

group and the Chrna7 (table 3). There was no observed difference between control and Chrna7 responses to vagal stimulation throughout the frequency range used ($p>.05$ by ANOVA), suggesting that the α7 subunit did not play an obligatory role at either spectrum of parasympathetic function (resting or high activity), see Figure 5.

Discussion.

Previous studies have provided both direct and indirect evidence for a functional role of α7 containing receptors in autonomic function at the cellular level. Studies in α7-deficient mice have indicated that baroreflex stimulation of the parasympathetic limb does not display altered parasympathetic function, but that stimulation of the sympathetic limb demonstrates abnormal sympathetic responses (7). The possibility has remained however, that alpha 7 subunits play a role in resting autonomic tone rather than in stimulated activity, and the possibility of some modulatory role on autonomic function has remained. In this study, we have shown that the α7 nAChR subunit is not required for parasympathetic modulation of heart rate in the resting state or under artificial controlled stimulation of vagal pathways to the heart throughout a wide stimulation range.

Resting HR and PSD profiles were not significantly different between the groups of mice suggesting that either the alpha 7 subunit does not play a role in resting autonomic tone, or that its role is replaced by a different subunit due to redundancy of nAChR subunits. We believe the latter is not a likely explanation, since despite the fact that many different subunits can form functional pentamers, changes in single subunits results in significantly different biophysical properties of the functional channel as reviewed by Role and Berg

(19). A change in subunit composition should therefore manifest itself in different "dose-response" curves such as those performed in the vagus nerve stimulation studies. Furthermore, it is unlikely that the α7 subunit forms heteropentamers with other subunits at neuronal synapses since in the autonomic nervous system, α7 subunits seem to form homomeric pentamers (6). However, we cannot exclude entirely the possibility that otherwise "redundant" α subunits might be utilized in the absence of α7 subunits.

An intriguing finding in our study is the lack of difference between normal and α7 deficient mice in HR responses following single and double autonomic blockade. Despite abnormal baroreflexes in the sympathetic limb as described previously (7), there was no detectable difference in resting HR or the responses to autonomic blockade in α7 deficient mice. Although blood pressure was not recorded in our studies and we cannot make any conjectures regarding BP, HR at rest and after individual and combined autonomic blockade was similar in both normal and alpha7 deficient mice. Specifically, propranolol did not change HR. This suggests that the α7 subunit is not required for sympathetic tone at rest or that there is very little sympathetic tone at rest in the mouse. The former interpretation is commensurate with previous findings by others showing that blockade of alpha7 containing receptors results in functional changes only at high levels of stimulation (3). This also fits with the anatomical studies that have shown that the α7 subunit is located at the periphery of synapses where it may play a role in saturation of the synaptic cleft with neurotransmitter at high levels of stimulation (3; 8; 21). The interpretation that there was low sympathetic tone at rest in our mice is possible however. The failure to induce bradycardia after propranolol supports the notion that there is little

resting sympathetic tone to the heart in the mouse at rest. This interpretation is reinforced by the finding that there was significant bradycardia with propranolol following blockade of parasympathetic tone with atropine first (see Figure 4A). In this situation, propranolol prolonged R-R by ~20 msec suggesting blockade of parasympathetic tone uncovers/disinhibits sympathetic outflow, possibly by removal of vagal inhibition of sympathetic nerve terminals as described by Levy and coworkers (12). It also confirmed that we were able to detect changes when they were present.

The lack of bradycardia following propranolol in our study is in disagreement with a number of previous studies (9; 15) which have shown a moderate bradycardia of approximately 80 beats following the same dose of propranolol. The discrepancy between our findings and those in earlier literature may lie in the fact that previous studies looking at heart rate control in rodents were under dissimilar conditions involving different awake/sleep states of the animals and less acclimatization to the recording environments. Under these conditions, animals would have higher resting sympathetic tone due to the normal stress/activity response. A review of reports measuring baseline HR in male C57BL/6J mice by the means of telemetry shows an average HR of 577 bpm, well above what we have recorded. A possible explanation for this difference is that our animals were recorded while at rest and following environmental conditioning for extended periods to ensure a "low-stress" environment. The lower heart rates in our mice compared to those previously studied (470 bpm *versus* 650 bpm) (9), using the same dose of propranolol, support the notion of a "low-stress" environment. Nonetheless, in our studies, there were no consistent differences in HR responses to sympathetic blockade

between normal and α7 deficient mice. It is important to consider however, that although our recording conditions were of low sympathetic tone, abnormalities may exist in the α7 deficient mice, which were not detectable by our methods. This point is highlighted by previous reports which demonstrate sympathetic dysfunction in α7 deficient mice undergoing baroreflex stimulation (7). In our study, there was a decrease in R-R variance that was seen after propranolol administration that was attenuated in the Chrna7 mice. This difference did not reach statistical significance, but may represent a real difference between the two groups. In this scenario, there would be no significant difference in heart rates or variance after propranolol because resting sympathetic tone is low, but under conditions of increased sympathetic tone, we may be able to detect larger differences. The finding of a small decrement in variance after propranolol in the Chrna7 mouse group may point to defective sympathetic modulation in this group and should be interpreted cautiously. We also did not detect any differences between the wildtype mice and C57BL/6J mice in resting HR, response to medications administered or PSD, suggesting that the C57BL/6J can be used as a control for the α7 deficient mice for studies of resting HR.

These findings are important for a number of reasons. These studies show that it may be possible to differentiate between sympathetic and parasympathetic pathways to the heart at the ganglionic level rather than at the end-organ receptor (beta adrenergic versus muscarinic receptors). Blockade of sympathetic baroreflex function in the sympathetic limb may be possible by specific antagonists against α7 subunits since α7 deficient mice display abnormal sympathetic baroreflexes (7). We have demonstrated here that blockade

of α7 subunits would not be likely to affect parasympathetic function. The implications for such findings are potentially very exciting for clinical relevance. If pharmacologic discrimination between sympathetic and parasympathetic function is possible at the ganglion, one could theoretically block sympatho-excitation while leaving parasympathetic function and end-organ receptors in both limbs intact. Furthermore, stimulation of parasympathetic pathways in conditions ranging from heart failure, to ageing, to hypertension may provide means to alter sympatho-vagal balance and reduce risk of sudden cardiac death since chronic sympatho excitation and vagal withdrawal have been associated with increased risk of malignant arrhythmias (11; 13; 16). Discrimination of different subunits at each ganglion could provide a target for development of agonists to stimulate parasympathetic function while blocking sympathetic activity. Perhaps more exciting is the possibility that different organ targets utilize different subsets of nAChR subunits in efferent pathways. This would enable one to stimulate parasympathetic function to the gastrointestinal tract to increase motility while not affecting heart rate. Such differentiation of ganglionic nAChR is completely theoretical at this stage, however the diversity of nAChR subunits and their dramatic plasticity in response to a plethora of cellular signals makes such a concept feasible and worthy of further investigation.

Acknowledegments

This research was supported by a Department of Veterans Affairs Merit Review grant to Mark E. Dunlap, and a University of Milan FIRST 2002 grant to Nicola Montano.

Reference List

1. **Alkondon M and Albuquerque EX**. Initial characterization of the nicotinic acetylcholine receptors in rat hippocampal neurons. *J Recept Res* 11: 1001-1021, 1991.

2. **Bibevski S, Zhou Y, McIntosh JM, Zigmond RE and Dunlap ME**. Functional nicotinic acetylcholine receptors that mediate ganglionic transmission in cardiac parasympathetic neurons. *J Neurosci* 20: 5076-5082, 2000.

3. **Chang KT and Berg DK**. Nicotinic acetylcholine receptors containing alpha7 subunits are required for reliable synaptic transmission in situ. *J Neurosci* 19: 3701-3710, 1999.

4. **Cuevas J and Berg DK**. Mammalian nicotinic receptors with alpha7 subunits that slowly desensitize and rapidly recover from alpha-bungarotoxin blockade. *J Neurosci* 18: 10335-10344, 1998.

5. **Dias D, Viana P, de M, Fazan R, Jr., Ruscone TG, Porta A, Malliani A, Salgado HC and Montano N**. Intravenous amiodarone modifies autonomic balance and increases baroreflex sensitivity in conscious rats. *Auton Neurosci* 95: 88-96, 2002.

6. **Drisdel RC and Green WN**. Neuronal alpha-bungarotoxin receptors are alpha7 subunit homomers. *J Neurosci* 20: 133-139, 2000.

7. **Franceschini D, Orr-Urtreger A, Yu W, Mackey LY, Bond RA, Armstrong D, Patrick JW, Beaudet AL and De Biasi M**. Altered baroreflex responses in alpha7 deficient mice. *Behav Brain Res* 113: 3-10, 2000.

8. **Jacob MH and Berg DK**. The ultrastructural localization of alpha-bungarotoxin binding sites in relation to synapses on chick ciliary ganglion neurons. *J Neurosci* 3: 260-271, 1983.

9. **Jumrussirikul P, Dinerman J, Dawson TM, Dawson VL, Ekelund U, Georgakopoulos D, Schramm LP, Calkins H, Snyder SH, Hare JM and Berger RD**. Interaction between neuronal nitric oxide synthase and inhibitory G protein activity in heart rate regulation in conscious mice. *J Clin Invest* 102: 1279-1285, 1998.

10. **Just A, Faulhaber J and Ehmke H**. Autonomic cardiovascular control in conscious mice. *Am J Physiol Regul Integr Comp Physiol* 279: R2214-R2221, 2000.

11. **La Rovere MT, Bigger JT, Jr., Marcus FI, Mortara A and Schwartz PJ**. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac

mortality after myocardial infarction. ATRAMI Investigators. *Lancet* 351: 478-484, 1998.

12. **Levy MN and Zieske H**. Autonomic control of cardiac pacemaker activity and atrioventricular transmission. *J Appl Physiol* 27: 465-470, 1969.

13. **Malliani A and Montano N**. Emerging excitatory role of cardiovascular sympathetic afferents in pathophysiological conditions. *Hypertension* 39: 63-68, 2002.

14. **Malliani A, Pagani M, Lombardi F and Cerutti S**. Cardiovascular neural regulation explored in the frequency domain. *Circulation* 84: 482-492, 1991.

15. **Mansier P, Medigue C, Charlotte N, Vermeiren C, Coraboeuf E, Deroubai E, Ratner E, Chevalier B, Clairambault J, Carre F, Dahkli T, Bertin B, Briand P, Strosberg D and Swynghedauw B**. Decreased heart rate variability in transgenic mice overexpressing atrial beta 1-adrenoceptors. *Am J Physiol* 271: H1465-H1472, 1996.

16. **Mark AL**. Sympathetic dysregulation in heart failure: mechanisms and therapy. *Clin Cardiol* 18: I3-I8, 1995.

17. **Nakano J**. Effects of atrial and ventricular tachycardias on the cardiovascular dynamics. *Am J Physiol* 206: 547-552, 1964.

18. **Orr-Urtreger A, Goldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, Dani JA, Patrick JW and Beaudet AL**. Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. *J Neurosci* 17: 9165-9171, 1997.

19. **Role LW and Berg DK**. Nicotinic receptors in the development and modulation of CNS synapses. *Neuron* 16: 1077-1085, 1996.

20. **Rubini R, Porta A, Baselli G, Cerutti S and Paro M**. Power spectrum analysis of cardiovascular variability monitored by telemetry in conscious unrestrained rats. *J Auton Nerv Syst* 45: 181-190, 1993.

21. **Shoop RD, Martone ME, Yamada N, Ellisman MH and Berg DK**. Neuronal acetylcholine receptors with alpha7 subunits are concentrated on somatic spines for synaptic signaling in embryonic chick ciliary ganglia. *J Neurosci* 19: 692-704, 1999.

22. **Ullian EM, McIntosh JM and Sargent PB**. Rapid synaptic transmission in the avian ciliary ganglion is mediated by two distinct classes of nicotinic receptors. *J Neurosci* 17: 7210-7219, 1997.

23. **Yu CR and Role LW**. Functional contribution of the alpha7 subunit to multiple subtypes of nicotinic receptors in embryonic chick sympathetic neurones. *J Physiol* 509.3: 651-665, 1998.

24. **Zhang ZW, Vijayaraghavan S and Berg DK**. Neuronal acetylcholine receptors that bind alpha-bungarotoxin with high affinity function as ligand-gated ion channels. *Neuron* 12: 167-177, 1994.

Table 1. Heart rates under experimental conditions

Group	Baseline	n	Atropine	n	Propranolol	n	Hexameth.	n
Control	471±21	16	667±16	5	444±32	5	626±21	6
Wildtype	446±24	12	630±31	4	530±31	4	636±27	4
Chrna7	442±18	22	606±24	7	442±27	7	579±31	7

Table 1. Data are given as mean beats per minute ± SE. n = number of recordings from N= 5 control (C57BL/6J), 4 wildtype (C57BL/6-129S), and 8 Chrna7 mice. Values are from recordings taken when each drug was given first in the protocol.

Table 2. Spectral analysis of heart rate variability summary data for control (C57BL/6J), wildtype (C57BL/6-129s) and Chrna7 mice.

GROUP	Mean	Variance	LFHz	LF	LFnu	HFHz	HF	HFnu	LF/HF	Resp.
Control	141.3	124.3	0.4	49.2	40.4	2.0	48.8	41.4	5.2	2.3
SE	8.8	68.6	0.1	27.4	14.8	0.2	40.6	2.0	4.5	0.2
WildType	143.3	237.6	0.4	148	55.3	2.2	60.4	28	9.3	2.3
SE	16.8	99.8	0	98.7	15.1	0.2	36.6	11.6	9.2	0.5
Chrna7	127.2	121.9	0.4	50	39.5	2.21	34.8	36.6	3.3	2.2
SE	11.1	49.8	0.1	30.6	17.4	2.2	27.5	14.8	2.3	0.2

Table 2. Data are given as measurement of sinus cycle length; mean ± SE, LFHz = low frequency component in hertz, LF=low frequency, LFnu = low frequency normalized units, HFHz = high frequency component in hertz, HF = high frequency, HFnu = high frequency in normalized units, Resp = respiratory frequency in hertz. N=5 control (C57BL/6J), 4 wild type (C57BL/6-129s), and 8 Chrna7.

Table 3. Baseline and vagal stimulation R-R intervals in control vs. Chrna7mice.

Stim. Level	Control Baseline RRI	Control Stimulation RRI	Chrna7 Baseline RRI	Chrna7 Stimulation RRI
5 Hz	213±9	323±32	280±26	338±35
10 Hz	215±8	397±30	275±23	422±26
15 Hz	216±9	474±38	275±24	486±38
20 Hz	216±10	523±42	288±23	525±44

Table 3. Data are given as mean R-R interval (RRI) in msec ± SE. Change in R-R is shown graphically in Figure 5, ($p>0.05$) for all stimulations. N= 7 control (C57BL/6J) & 6 Chrna7 mice.

Figure 1.

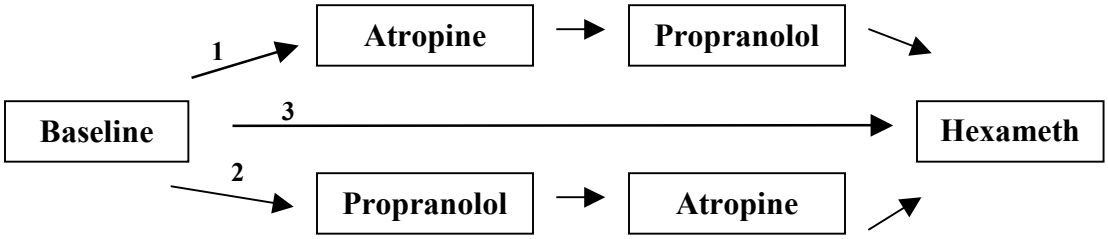

Figure 1. Protocol for conscious studies with autonomic blockade. Animals were randomly slected to undergo either protocol 1, 2, or 3 on testing day. Only one protocol was performed in any given day with at least 48 hr rest period between repeat studies.

Figure 2(A).

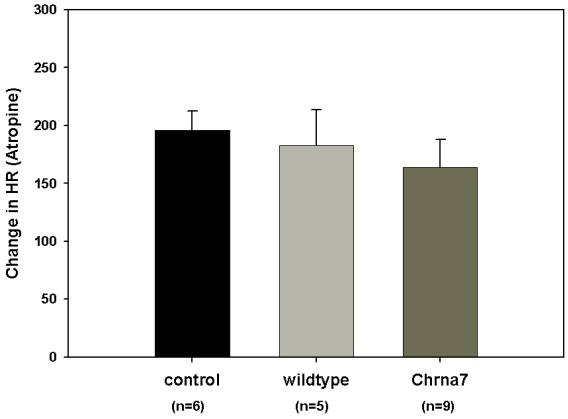

Figure 2(B).

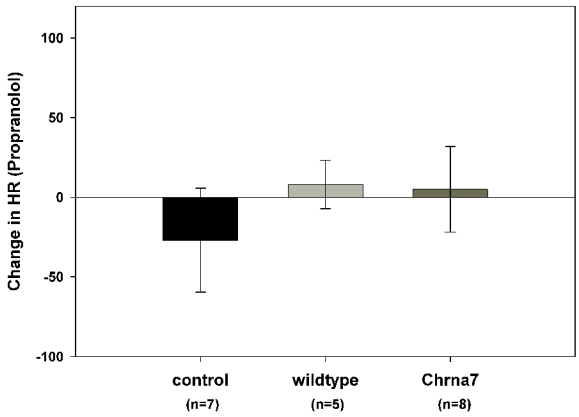

Figure 2(C).

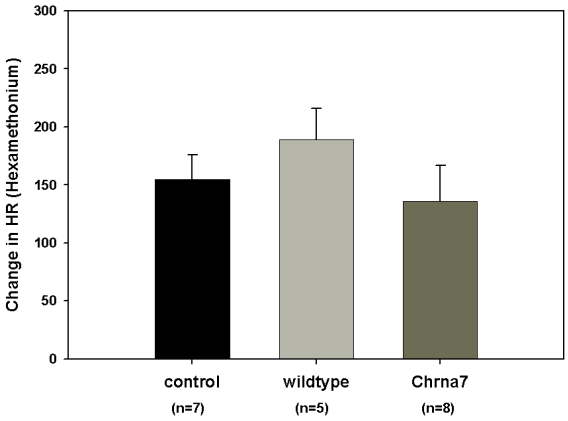

Figure 2. Change in HR from baseline (beats per minute) following autonomic blockade. Atropine sulfate (A), propranolol (B), or hexamethonium (C) in control (C57BL/6J), wildtype (C57BL/6-129s), and Chrna7 mice. n = number of different mice. There were no statistically significant differences ($p>.05$, t-test) between the groups in HR responses to any of the pharmacologic agents tested.

Figure 3.

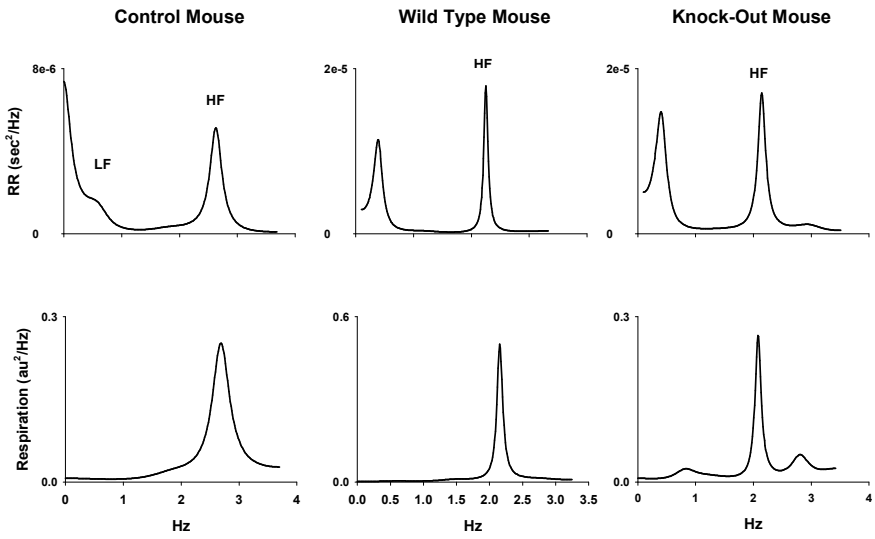

Figure 3. Power Spectral Analysis for control, wildtype and α7 deficient mice. Note the tight coupling of the HF component with the respiratory frequency in each group representing parasympathetic tone, and an LF component representing sympathetic tone at rest. There were no statistical differences between any of the groups ($p>0.05$, t-test). N=6 control (C57BL-6J), 4 wildtype (C57Bl-6/129s) and 8 Chrna7.

Figure 4A. **Figure 4B.**

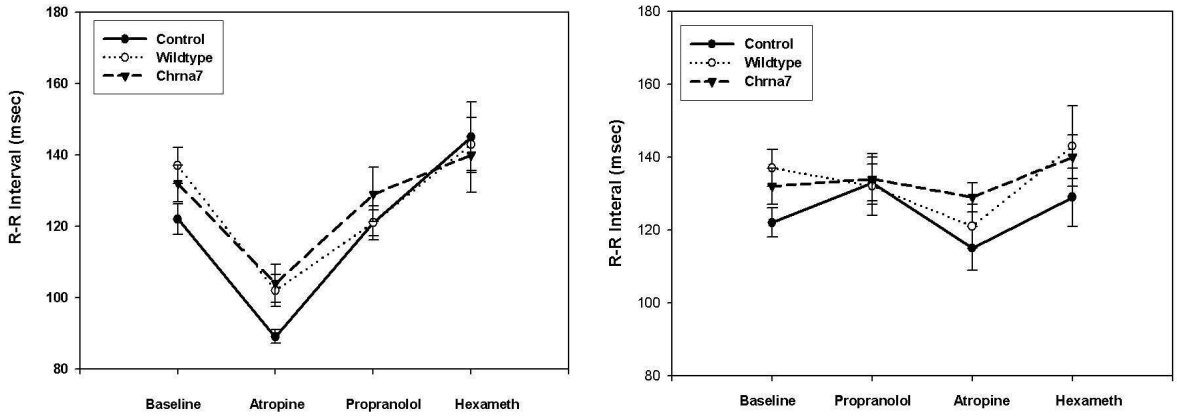

Figure 4. R-R Interval after sequential addition of pharmacologic agents. Atropine caused a significant tachycardia (shorter R-R interval) equally in each group confirming prominent parasympathetic tone at rest in each group. Note that propranolol caused a bradycardia if animals had received atropine previously (A), but did not induce a bradycardia when given first (B) suggesting that there was little sympathetic tone at rest. Hexamethonium caused a small bradycardia when given following both sequences that likely resulted from inhibition of co-transmitter release from the post ganglionic neuron, n = 7 animals for each group. There was no statistical significance between the groups at each data point (t-test $p>0.05$).

Figure 5.

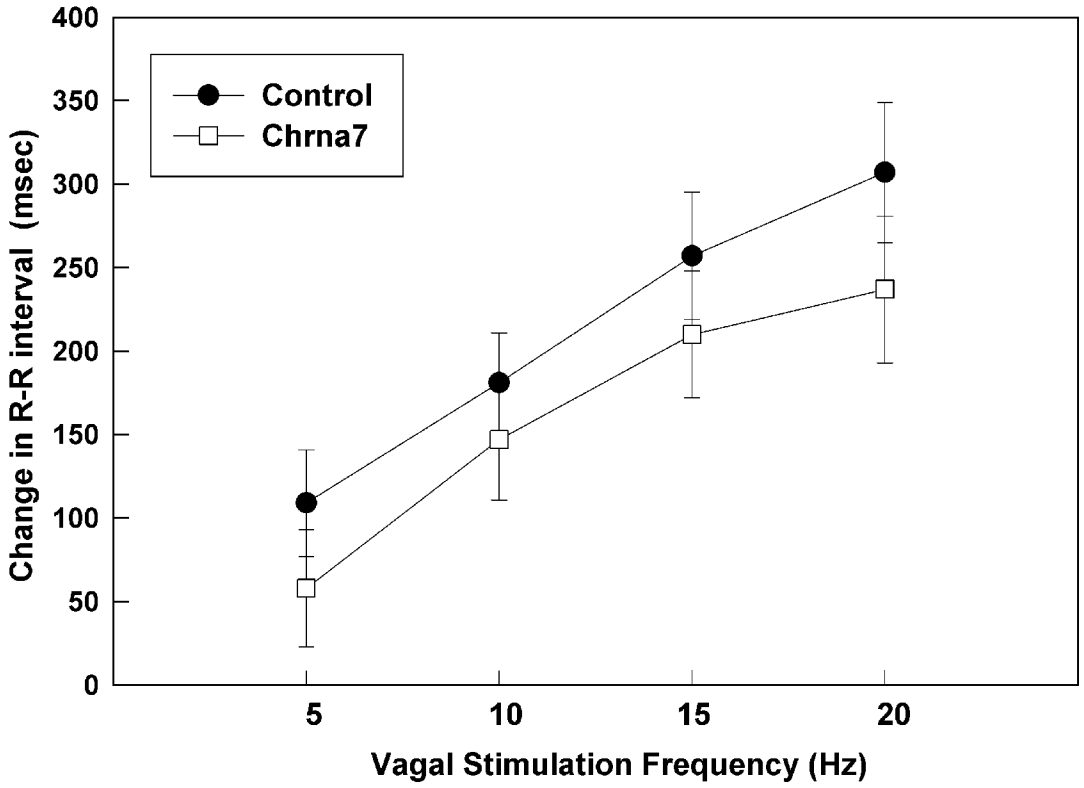

Figure 5. Change in R-R interval with vagus nerve stimulation at different frequencies. There was no observed difference between control and Chrna7 mice responses to nerve stimulation throughout the range of stimulations ($p>0.05$, ANOVA).