Concomitant assessment of heart rate and behavior in freely moving mice

Oliver Stiedl, Anton W. Pieneman, Jiun Youn, and René F. Jansen

Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, The Netherlands, oliver@cncr.vu.nl

Heart rate (HR) and its adjustment are affected by different physiological conditions such as sleep, physical activity and the emotional state. The instantaneous beat-by-beat fluctuation of heartbeats provides important information about the physiological state of the brain. While the precise origin of beat-by-beat HR variability has not been identified clearly, measures of HR dynamics provide critical clues for the assessment of physiological versus pathological states. By combining autonomic measures in mice during fear learning and memory experiments with advanced non-linear data analysis, we developed a combination of methods that is useful to compare mouse and human HR dynamics for a translational approach [1]. The translation of results is a prerequisite for the development of adequate animal models of affective disorders to improve our understanding of causal mechanisms involved in the comorbidity of emotional and cardiac disorders.

Current telemetry systems are able to monitor ECG, blood pressure and body temperature in mice. However, many commercially available systems provide only averaged HR values. We have mainly focused on HR measurements derived from ECG recordings since (i) HR can be obtained with high precision at high data sampling rates, and (ii) the dynamical adjustment of HR, as opposed to blood pressure, is under direct neuroautonomic control with fast adjustment dynamics in both mice and man. A first important issue is to determine the normal physiological range of HR of mice under baseline stress-free conditions in the home cage. The impact of different recording techniques and experimental conditions on HR is described elsewhere [2]. Long-term HR recordings reveal baseline HR levels in C57BL/6J and C57BL/6N mice predominantly in the range of 550-600 beats per minute [bpm]. With these values in mind it is not surprising that the interpretation of effects of pharmacological interventions with baseline HR values of 720 bpm have led to the misconception that HR in mice is not under parasympathetic control while the sympathetic tone predominates, while our results show exactly the opposite. Experiments in rodents so far have failed to convincingly show the effect of the anxious state on HR dynamics because the experimental conditions under which HR changes have been measured were generally inadequate to assess phasic changes. Any intervention with the experimental animal immediately before the actual anxiety test will confound the subsequent autonomic measure of interest. Thus, behaviorally established anxiety tests such as the dark-light test, elevated plus maze, open field test and novelty exposure are unsuited for measurement of fast HR adjustments because they require handling. Any exposure to a novel environment including a plain change of the home cage serves as unconditioned stressor and results in initial HR values close to 800 bpm representing the maximum physiological limit in this species. The recovery of HR to baseline values (550-600 bpm) is assumed to require 1-2 hr in C57BL/6J mice [3]. While novelty exposure is useful to assess maximum HR values under physiological conditions, it is not expected to reflect HR changes that would occur normally when starting out from baseline values. Therefore it is crucial to change strategies by offering the animal a choice for exploring a novel environment from a home base without interference by the experimentalist. A first attempt has been made in this direction, however,

without being able to remove all aversive experimental interference as indicated by highly elevated (> 700 bpm) baseline HR values [4]. Based on this complication and the need for refined experimental conditions, our focus so far has been the investigation of HR responses during expression of fear conditioned to an auditory cue that can be tested in the home cage in combination with genetic or pharmacologic interventions [5-7]. Re-exposure of mice to a tone serving as conditioned stimulus that has been previously paired with aversive stimulus such as foot shock elicits a fear response that results in a profound increase of HR close to maximum physiological limits of ~800 bpm [1,5-7]. Furthermore, startle experiments have been performed under habituated conditions rather than under semi-restrained conditions in conventional startle systems that show that startle responses elicit phasic HR changes that do not suggest a strongly aversive effect [2]. The combination of behavioral and autonomic measurements without intervention by the experimenter is required to further characterize the relation between HR and physical activity in various behavioral aspects in a more thorough way.

A dysfunctional central autonomic system is expected to contribute to elevated risk of cardiac mortality. While the central autonomic pathways have been anatomically characterized in considerable detail, their functional significance is currently still poorly understood because of (i) non-physiologic experimental conditions, (ii) inadequate and insensitive analytical methods, and (iii) lack of spatial and temporal precision of interventions. The appropriate combination of state-of-the-art methods applied in basic research using valid animal models will improve our understanding of the functional significance of central autonomic pathways and their receptors for a translational approach to identify mechanisms underlying human cardiac risk in affective disorders. This translational approach is expected to improve diagnostics and facilitate therapeutic interventions in the clinical setting eventually providing for a stronger linkage between cardiology, neuroscience, psychology and psychiatry (neurocardiology). Aim of the presentation is to provide an overview on current methods and techniques and to give a perspective for future needs in combining behavioral and autonomic measurements.

References

1. Meyer, M., Stiedl, O. (2003). Self-affine fractal variability of human heartbeat interval dynamics in health and disease. *European Journal of Applied Physiology*, **90**, 305-316.
2. Tovote, P., Meyer, M., Pilz, P.K.D., Ronnenberg, A., Ögren, S.O., Spiess, J., Stiedl, O. (2005). Dissociation of temporal dynamics of heart rate and blood pressure responses elicited by conditioned fear but not acoustic startle. *Behavioral Neuroscience*, **119**, 55-65.
3. Stiedl, O., Tovote, P., Ögren, S.O., Meyer, M. (2004). Behavioral and autonomic dynamics during contextual fear conditioning in mice. *Autonomic Neuroscience: Basic and Clinical*, **115**, 15-27.
4. Depino, A.M., Gross, C. (2007). Simultaneous assessment of autonomic function and anxiety-related behavior in BALB/c and C57BL/6 mice. *Behavioural Brain Research*, **177**, 254-260.

5. Tovote, P., Meyer, M., Ronnenberg, A., Ögren, S.O., Spiess, J., Stiedl, O. (2005). Heart rate dynamics and behavioral responses during acute emotional challenge in corticotropin-releasing factor receptor 1-deficient and corticotropin-releasing factor-overexpressing mice. *Neuroscience*, **134**, 1113-1122.

6. Meyer M, Stiedl O (2006) Fractal rigidity by enhanced sympatho-vagal antagonism in heartbeat interval dynamics elicited by central application of corticotropin-releasing factor in mice. *Journal of Mathematical Biology*, **52**, 830-874.

7. Stiedl, O., Misane, M., Koch, M., Pattij, T., Meyer, M., Ögren, S.O. (2007). Activation of the brain 5-HT2C receptors causes hypolocomotion without anxiogenic-like cardiovascular adjustments in mice. *Neuropharmacology*, **52**, 949-957.