Respiration and Emotion: How and where are they linked?

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Thesis submitted for PhD (Human Physiology)

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The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made available in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act of 1968.*

* Part of the experiment in Chapter 1 (10 animals implanted with telemetric transmitters for cardiac assessment) was performed as part of my Honours project.

The data was subsequently reanalyzed, combined with data obtained during my PhD project and submitted for publication, which was written as part of my PhD project.

I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

The following publications are included as part of the thesis:

Bondarenko, E., Hodgson, D.M. and Nalivaiko, E. (*submitted*). Respiratory arousal is an autonomic and a behavioral index of anxiety in rats.

Bondarenko, E., Beig, M.I., Hodgson, D.M., Braga, V. and Nalivaiko, E., 2015. Blockade of the dorsomedial hypothalamus and the perifornical area inhibits respiratory responses to arousing and stressful stimuli. Am. J. Physiol. Regul. Integr. Comp. Physiol., doi: 10.1152/ajpregu.00415.2014.

Bondarenko, E., Hodgson, D.M., Nalivaiko, E., 2014. Amygdala mediates respiratory responses to sudden arousing stimuli and to restraint stress in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 306, R951-R959, doi: 10.1152/ajpregu.00528.2013.

Bondarenko, E., Hodgson, D.M., Nalivaiko, E., 2014. Prelimbic prefrontal cortex mediates respiratory responses to mild and potent prolonged, but not brief, stressors. Respir. Physiol. Neurobiol., doi: 10.1016/j.resp.2014.07.009.

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I, Eugene Nalivaiko, attest that Research Higher Degree candidate Evgeny Bondarenko performed experiments, analyzed data, interpreted results of experiments, prepared figures, drafted, edited and revised the manuscript "Respiratory arousal is an autonomic and a behavioral index of anxiety in rats."

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CONTENTS:

ABSTRACT

The link between respiration and emotions is well documented in humans, but animal studies addressing this issue are limited. The current project aims to systematically examine respiratory responses to stressors and stimuli of various intensity and length in rats, compare and correlate these responses with cardiac responses and behavioural indices of anxiety and lastly to investigate central neuronal pathways that mediate them. In the first chapter we show that respiratory responses to brief changes in arousal are more sensitive than traditionally used cardiac responses. Furthermore, respiration during the novelty stress is highly correlated with behavioural indices of anxiety in rats. The subsequent three chapters investigate involvement of the dorsomedial hypothalamic area, the amygdala and the prelimbic prefrontal cortex in mediating respiratory responses to brief and prolonged stimuli of various intensities. This is achieved by examining the effects of inhibition of the target areas with a microinjection of $GABA_A$ agonist muscimol. Inhibition of the dorsomedial hypothalamic area abolished respiratory response to the novelty and restraint stress protocols and also significantly inhibited responses to the brief acoustic stimuli. Blockade of the amygdala significantly inhibited responses to the high-intensity stressors of both brief (70-90dB acoustic stimuli) and prolonged (restraint) duration, but had little effect on responses to the low-intensity stimuli (novelty stress and 40-70dB acoustic stimuli). Lastly, inhibition of the prelimbic prefrontal cortex significantly inhibited the respiratory responses to the prolonged stressors (restraint and novelty stress), but had no effect on responses to brief stimuli (acoustic stimuli). Overall, our findings suggest that (i) assessment of respiratory response can be used as a novel index of anxiety in rats; (ii) respiratory rate is more sensitive to changes in arousal than traditionally used heart rate, which has implications for the definition of an orienting response as it is currently defined only in terms of heart rate. Lastly, (iii) we show that the dorsomedial hypothalamic area and the amygdala have critical roles in mediating stress-induced respiratory changes.

OVERVIEW

Links between respiration and emotion were demonstrated in humans a century ago (Feleky, 1914); yet very little animal research has been done on this topic. Our recent observations suggest that respiratory rate is closely related to animals' anxiety state as it is sensitive to anxiolytic drug diazepam (Nalivaiko et al., 2011) and is elevated in animals bred for high anxiety behaviour (Carnevali et al., 2013). Yet, to date, there was no systematic investigation of respiratory changes accompanying brief and prolonged changes in physiological arousal and central neuronal structures that mediate such changes.

The aim of this thesis is to systematically investigate and document respiratory responses to stimuli and stressors of various intensities and duration and to investigate underlying central pathways that mediate these responses. In the 1st Chapter we compare and correlate such responses with cardiac responses, a traditionally used index of autonomic activation, and also with behavioural measures of anxiety as assessed by the Elevated Plus Maze, a standard behavioural test for anxiety. Our results indicate that in rats respiratory responses to brief stimuli have a lower threshold of activation than cardiac responses. This finding has implications for the definition of an orienting response (i.e. a physiological response that indicates that an animal has paid attention to a stimulus). Furthermore, respiration during novelty stress was highly significantly correlated with behavioural measures of anxiety as assessed by the Elevated Plus Maze. This finding suggests that assessment of respiration can potentially be used as a measure of anxiety in animals.

The subsequent chapters investigate central neuronal pathways that mediate such respiratory responses to various stimuli. In particular, we investigate involvement of the dorsomedial hypothalamic area (Chapter 2), the amygdala (Chapter 3) and the prelimbic prefrontal cortex (Chapter 4). Inhibition of the dorsomedial hypothalamic area almost completely abolished respiratory responses to brief acoustic stimuli and abolished an increase in respiration during prolonged stressors. Previous studies suggest that the dorsomedial hypothalamic area is the integrative centre of central autonomic information (Buijs and Van

Eden, 2000); inhibition of this area abolishes increases in heart rate and arterial pressure in response to stress (DiMicco et al., 1996). Our finding extends this previous knowledge and suggests that the dorsomedial hypothalamic area similarly integrates central respiratory command.

Both the amygdala and the prelimbic prefrontal cortex have projections to the dorsomedial hypothalamic area (Dampney et al., 2008). Inhibition of the amygdala significantly inhibited respiratory responses to the high intensity stimuli and stress, both brief and prolonged, which is in line with its well-documented role in fear and emotional processing. Interestingly, inhibition of this area did not affect respiratory responses to low-intensity stimuli and stress (i.e. those that evoke an orienting response). Inhibition of the prelimbic prefrontal cortex attenuated respiratory arousal during presentation of prolonged stressors – novelty stress and restraint, but did not affect generation of respiratory responses to brief acoustic stimuli. The exact role of the prelimbic prefrontal cortex in autonomic processing is still poorly understood; previous studies suggest that this area modulates cardiovascular changes during stress and baroreflex (Resstel and Corrêa, 2005). Our findings suggest that the prelimbic prefrontal cortex also modulates respiratory changes during stress by modulating the overall level of autonomic arousal; however, it does not affect brief changes in autonomic arousal.

The findings of the current thesis propose that respiratory activation can be used a non-invasive measure of autonomic activation in rats. Also, assessment of respiration during novelty stress can be used as a locomotion-independent measure of anxiety. This is of special merit to preclinical psychopharmacology field as all existing anxiety tests are based on animals' ambulation. The thesis further describes relative contribution of three central neuronal structures – the dorsomedial hypothalamic area, the amygdala and the prefrontal cortex – to central processing of respiratory activation in response to brief and prolonged stressors of varying intensities.

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