A Novel Task for Evaluating Anticipatory Postural Behaviour in Rats

Daniel I. Aponte

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By: Daniel I. Aponte

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complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final Examining Committee:

Dr. Andreas Bergahl	Chair
Dr. Peter Shizgal	Examiner
Dr. Nancy St-Onge	Examiner
Dr. Richard Courtemanche	Supervisor

Approved by: *Dr. Simon Bacon*

Graduate Program Director

October 2013

Joanne Locke

Dean of Faculty of Arts and Science

Abstract

A variety of human and animal postural tasks are commonly used to describe the different strategies used to cope with a perturbation of the base of support. Rodent research on posture is generally limited to tasks which test dexterity and coordination more than posture per se. We designed a novel task in which a conditioning stimulus tone was coupled to a platform drop in order to entrain anticipatory postural behaviour. Rats were conditioned using delay-conditioning, which consisted of a 2 s stimulus tone coupled to the drop of a moveable platform. The platform was fitted with a force plate built with a clear acrylic sheet and 4 load cells. This force plate measured ground reaction forces which were used to calculate center of pressure (CoP), CoP displacement, CoP excursion and CoP variability. Video of the animal behaviour was recorded from underneath the platform and used to calculate the orientation of the animal. It was found that trained rats increased their CoP displacement, CoP excursion and CoP variability in response to the stimulus tone, before the platform drop. Trained animals consistently moved toward the back of the platform upon hearing the tone, indicating acquisition of the desired conditioned response, and understanding of the parameters of the task. There were no training-related changes to orientation variables, but the animals demonstrated a preference for facing the interior of the platform. This is the first step in describing rat behaviour in an anticipatory behaviour paradigm.

Acknowledgements

I would first like to preface this by confessing that first and foremost, this entire project began as a selfish need to satisfy my curiosity. Over the years of taking public transit, be it the train or metro, I had noticed a most peculiar thing; my body shifted and moved to accommodate the movement of the train. What really intrigued me was that I anticipated the timing of the acceleration of the metro from the closing of the metro doors. After asking some friend about it, I generally got 2 answers: either they had noticed it and that they likened it to walking up to an out-of-service escalator or, to my dismay, that they had never noticed it. Funnily enough, many of these people got back to me later affirming that they too had noticed it, but simply weren't aware of it until I brought it up.

I brought this idea to Richard one fateful February morning, who at first was struck by the simplicity of this task, but later relished at the possibilities it held: the novelty of the task, the translational nature of the task and the multiple levels of exploration possible, be it electrophysiology or model testing. To be honest, it was quite a gambit on both our parts; it was a high risk project that could, in the end, result in nothing. Neither one of us knew of the complications that would arise from this project nor was it in a field that was directly related to our previous experience (Richard having his formal training in electrophysiology and not rodent biomechanics and behaviour). For this I am grateful; few supervisors would have been open to the idea of embarking on such uncharted territory. Whether it was wisdom or folly will be determined by the following pages.

The multidisciplinary nature of this project made it such that I would not have been able to do it without the help of such a vast number of people from a range of backgrounds. In no particular order, I would like to thank Jon, Jen, Justin, Ariana and Shannon from our lab, Aldo Dissegna and Richard Allix for building the prototype and final version of the testing

apparatus and their technical help, Dr. Nadia Chaudhri for her suggestions regarding rodent conditioning and recommendations on the alcohol model I used, Dr. Barbara Woodside for her insight on rodent development and for the use of her animals, Claudia Frate for showing me everything I now know about handling and rearing pups, Dr. Rick Gurnsey for his MATLAB help, Dr. Nancy St- Onge for her help with some of my biomechanics problems and agreeing to be on my committee, Dr. Peter Shizgal for his astute suggestions, which resulted in a more complex but more robust description of animal behaviour, and for agreeing to be on my committee, Dr. Andreas Bergdahl for far too much help for me to do justice in just a few lines of text, Patrice Desaulniers and Robert Panenic for always keeping me in good spirits, my friends and my family for always being there. I would finally like to thank Emilie for being my cornerstone for the past 2 challenging years.

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List of Abbreviations

CoP.....Center of Pressure

CoPDx .....Displacement of Center of Pressure in x-axis

CoPDy ..... Displacement of Center of Pressure in y-axis

CoPEx..... Excursion of Center of Pressure in x-axis

CoPEy..... Excursion of Center of Pressure in y-axis

CoPVx ..... Variability in Center of Pressure in x-axis

CoPVy ..... Variability in Center of Pressure in y-axis

CS..... Conditioned Stimulus

FASD .....Foetal Alcohol Spectrum Disorder

PD .....Post-natal day

PMRF .....Pontomedullary Reticular Formation

RMS .....Root Mean Square

US..... Unconditioned Stimulus

Introduction

Daily, we are faced with navigating through our environment, challenged by both unexpected and predictable disturbances of posture. Posture serves two main functions: to work against gravity and maintain balance, as well as to properly interface with the environment without losing a base of support (Massion, Ioffe et al. 1999). One of the main challenges for humans and animals is to control the displacement, the rotation and translation of their center of mass against external forces. In order to prevent falls, a variety of reactive and predictive strategies are used, each context-specific (Macpherson and Horak 2013). Human models of postural adaptation generally look at muscle synergies via electromyography, limb strategies via joint kinematics, or force and center of pressure changes using force platforms (Horak and Nashner, 1986; Stapley et al 1998). These studies have spanned from research into fall prevention in the elderly (Bhatt and Pai, 2008) to advancements in rehabilitation techniques for patients with a variety of conditions, both central and peripheral (Rossignol, 2000; Morton & Bastian, 2006, Bunday & Bronstein, 2008, Ooteghem et al, 2008).

In order to best prevent falls, repeated exposure to perturbations permit the identification of cues which allow their prediction. Recently, researchers investigated if individuals could transfer acquired improvements in the control of balance during walking obtained from training in a slip-simulation to improvements of walking in real-world slip situations. Bhatt et al. (2006) designed a task where subjects were trained to walk across a walkway fitted with a low-friction metal plate, free to move overtop a force plate, meant to simulate a slip. Subjects fitted with a safety harness were instructed to walk across the walkway at their preferred walking speed and that in the case of a slip, to try and recover and keep walking. After the first 10 practice trials, subjects performed a total of 37 test-trials

separated in slip/non-slip blocks. The first block consisted of 8 slip-trials and 3 non-slip trials, followed by a second block of 8 slip-trials and 3 non-slip trials and a final block of 15 trials with 8 randomly occurring slips. The subjects were never instructed which trials had slips in them, and the slips always occurred under the right leg of subjects. After this training protocol, subjects walked across a different surface. The first 3 trials were non-slip practice trials and the subsequent 6 were test trials where the subjects were instructed a slip could occur. The slip was induced by applying a lubricant to the floor in only 1 of the 6 test trials. Control subjects only performed the real-slip portion of the experiment with no slip training sessions. The results of the slip-simulation training session demonstrated that subjects improved their performance from block to block. Additionally, the results of trained and control subjects in the real-slip condition showed that trained subjects performed significantly better than controls, with improved stability results. All of the trained subjects were able to walk across the real-slip condition without falling, and all but one of the trained subjects managed to do so without losing their balance. The control group had a fall incidence of 38% and had a 100% loss of balance rate. The results of the control groups were comparable to the results of the first unexpected slip of the training session. These results suggest that improvements in gait and fall prevention are possible with training and that they can be translated to real-life situations (Bhatt and Pai, 2009).

Another example of posture and gait anticipation behaviour is the sensation one feels when walking up to an out-of-order escalator. Anecdotally, one can relate to the strange feeling of leaning forward in anticipation to stepping onto the escalator, expecting it to pull us forward. Although this compensation is not usually felt when walking onto working escalators, the incorrect compensation is felt because of the mismatch between the expectation of forward movement and the reality of the stationary belt. Surprisingly, this adaptation is triggered despite the visual indication that the belt is not moving. Inspired by

this, Reynolds and colleagues (2003) investigated this very phenomenon in the laboratory. Dubbed the 'broken escalator phenomenon', this group built a walkway with a moveable sled at the end of it. Healthy subjects were habituated to the walkway and moveable sled, which was kept stationary during these 10 practice trials. Subjects were then instructed that the sled would move away from them at a moderate speed (1.2 m/s) when they crossed a photo-beam trigger. They walked across the walkway and moveable sled a total of 20 trials. The baseline walking speed of subjects was recorded at 0.6 m/s and after the first 3 trials, it increased to 0.9 m/s. After the 20 moving trials, subjects were again asked to walk across the walkway but were explicitly instructed that the sled would not move. Despite this information, all subjects showed residual aftereffects in increased walking speed (0.71 m/s), increased forward trunk sway and increased electromyography activity in leg muscles for the first 2 trials. All variables quickly returned to baseline subsequent to this, but subjects reported being surprised by their behaviour, and feeling a similar sensation during the experiment as experienced in real life on escalators. The authors suggest this phenomenon is indicative of the dissociation between declarative and procedural systems, with the nervous system weighting experience and context more than declarative information (Reynolds and Bronstein, 2003). The results of these two studies indicate that although anticipatory postural adjustments are context-specific, the central nervous system is able to generalize from experience and apply it under different environmental conditions. Intuitively this makes sense, but these two paradigms recreate gait and posture disturbances inspired by anecdotally reported perturbations of daily-living in the laboratory, and measure related learning of adaptive posture control (Bhatt et al., 2006, Reynolds and Bronstein, 2003).

Research on postural adaptations in animals often takes place in the context of locomotor adaptation (Drew, 1988; Schepens, Stapley and Drew, 2008). As animals can rely on central pattern generators in gait (Grillner and Wallen, 1985, Kiehn, 2006), which can be

affected by spinal cord injuries (Rossignol, 2000; Kiehn, 2006), a more direct study of postural control focuses on compensatory mechanisms to cope with unexpected perturbations in cats (Stapley and Drew 2009). In a set of electrophysiology studies, Stapley et al. (2009) recorded the activity of pontomedullary reticular formation (PMRF) neurons of the brainstem of cats trained to stand on 4 independent force plate blocks (one under each paw). The trial consisted of a brief tone to signal trial commencement, followed by the random drop of one of the 4 blocks 2.5 s later. The authors note that the animal could not learn to anticipate which block would drop because it was always different and random, and the force plate data did not demonstrate any anticipatory shift. This perturbation produced robust initial reactive postural adjustments followed by stable three-legged posture on the remaining force plates. 92% of recorded PMRF neurons showed modified activity associated to the perturbation of at least one limb, and 81% of recorded neurons showed changes in activity associated to the perturbation of 2 or more limbs (Stapley and Drew, 2009). The activity was characterized by an initial high frequency short duration burst of activity associated to the reactive postural adjustment. The initial burst activity quickly subsided during the stable 3 legged-stance, but was higher than baseline measures. The authors suggest the PMRF as one of the contributors to the compensatory responses that restore equilibrium during unexpected postural disturbances (Stapley and Drew, 2009).

Locomotion has also been studied in rodents under challenging conditions. Tasks such as the rotarod or the parallel beam walking tests are able to separate various neuropathological models in mice and rats (Kennard and Woodruff-Pak 2011, Deacon 2013). However, these tasks primarily test animal coordination and dexterity and only indirectly postural control. The ladder-rung-walking test in rats was developed in order to describe skilled walking and measure parameters such as limb placement, stepping speed and coordination in an attempt to measure more biomechanical features of animal behaviour

(Metz and Whishaw, 2002). In this task, the rungs of the narrow walkway are placed at varying lengths of separation trial after trial to prevent the animals from learning the locations of the rungs. The animals traverse the walkway while a camera records their gait. Frame-by-frame analysis of the video is performed and the animal's gait is rated using different scoring systems. A foot-fault scale from 0-6 is used to score misteps and a forepaw score from 0-2 depending digit placement are used to rate the severity and type of mistake (Metz and Whishaw, 2002). Researchers investigated if this novel task was sensitive enough to discriminate between a range of neurological deficits and a group of old rats. They concluded that indeed, the ladder-rung-walking test is sensitive enough to discriminate between a variety of motor system or by aging. These studies demonstrate the validity of rodent tests to distinguish motor deficits, but do not directly address postural components, and certainly do not assess anticipatory posture behaviour.

When placed on a tilted surface, rats have been shown to brace and back away from the dropping end in order to avoid falling or slipping off the surface (Pellis and Pellis 2005). Knowing this specific and quantifiable behaviour, we expect a platform drop to elicit this reactive behaviour. One of the objectives of this project was to design and build a testing platform capable of measuring rat posture variables and be capable of challenging rat's base of support with this kind of tilted drop. Classical conditioning has been used to entrain desired behaviour in animals in a variety of tasks since the time of Pavlov. We hypothesize that rats can be conditioned to associate a stimulus tone (conditioned stimulus; CS) to a platform drop (unconditioned stimulus; US) that would then entrain anticipatory adjustments prior to the platform drop. The CS will provide useful information to the animal; as the animal walks around completely unrestricted on the platform, it will have a much better chance at staying on the platform if it can stop whatever it is doing, prepare for the tilt and brace. We predict that over the course of conditioning the rats would learn to use the CS to anticipate the US and

alter their behaviour to better cope with the demands of the perturbation. The previously described rodent tasks, while testing adaptation and coordination, do not directly use postural control variables and do not focus on the learning of anticipatory behaviour. The described cat task, which tests postural stability and control, does not have a component of anticipatory postural adjustment. The objectives of this project were to design and build a testing platform capable of measuring rat postural variables, and test rat anticipatory postural behaviour.

A Novel Task for Evaluating Anticipatory Postural Behaviour in Rats

Daniel I. Aponte and Richard Courtemanche

FRQS Groupe de Recherche en Neurobiologie Comportementale (CSBN)
Department of Exercise Science
Concordia University, Canada

Correspondence:
Richard Courtemanche, Ph.D.
FRQS Groupe de Recherche en Neurobiologie Comportementale/Center for Studies in Behavioral Neurobiology
Department of Exercise Science
SP-165-03, Richard J. Renaud Science Complex
7141 Sherbrooke Street West
Concordia University
Montréal (Qc) Canada H4B 1R6
richard.courtemanche@concordia.ca

Running title:
Rat anticipatory postural behaviour

Introduction

Posture serves two main functions: to work against gravity and maintain balance, as well as to properly interface with the environment without losing a base of support (Massion, Ioffe et al. 1999). For every movement that we produce, there are intrinsic and extrinsic postural disturbances that need to be negotiated in order to maintain balance. When postural disturbances can be temporally predicted, the nervous system can identify strategies that are most favourable and context-specific in order to cope with them. Human models of postural adaptation generally look at muscle synergies via electromyography, lower limb strategies via joint kinematics, or force and center of pressure changes using force platforms (Horak and Nashner 1986, Stapley, Pozzo et al. 1998). These studies have spanned from research into fall prevention in the elderly (Bhatt and Pai 2009) to advancements in rehabilitation techniques for patients with a variety of conditions (Rossignol 2000, Morton and Bastian 2006, Van Ooteghem, Frank et al. 2008), and even the prospect of increasing athletic performance (Misiaszek 2006).

With respect to anticipatory behaviour, Reynolds and Bronstein (2003) investigated the impact of previous experience and preconceived notions on gait. Dubbed the 'broken escalator phenomenon', subjects were asked to walk across a platform with a moveable sled at the end of it. After a set of practice and training trials where the sled moved away from the subjects, subjects were verbally instructed that the sled would no longer move in subsequent trials. Even so, the first 2 trials always showed residual aftereffects of an anticipated sled movement through forward trunk sway, increased walking speed and muscle electromyography (Reynolds and Bronstein 2003). Bhatt and Pai (2009) investigated if walking performance of subjects trained to walk across a moveable platform, meant to simulate a slip, would improve on a slippery floor. They showed that slip training on a moveable platform could indeed carry over to improvements in balance in trained subjects

compared to controls. Not only do these studies show evidence of anticipatory behavioural adaptations in humans, they also suggest that these context-specific adaptations can be generalized by the nervous system for optimal control of stability under different environmental conditions.

Research on postural adaptations in animals often takes place in the context of locomotion adaptation, especially in the cat (Drew 1988). As animals can rely on central pattern generators in gait (Grillner and Wallen 1985, Kiehn 2006), located in the spinal cord (Rossignol 2000), a more direct study of postural control focuses on compensatory mechanisms to cope with unexpected perturbations (Stapley and Drew 2009). These postural reactions rely on brainstem nuclei to show adaptive behaviour and the compensatory mechanisms to unpredictable perturbations (Stapley and Drew 2009).

Rodent research has looked at the locomotor system and how it performs under different conditions. There are tasks such as the rotarod, horizontal bars (or coat-hanger test), parallel bars and parallel beam walking tests which are able to separate various neuropathological models in mice and rats (Deacon 2013). However, these tasks primarily test animal coordination and dexterity but do not measure postural variables. Metz and Whishaw (2002) developed a more direct way to investigate rat gait and limb coordination called the ladder-rung-walking task. In this task, researchers modified the position of the rungs to tax the locomotor system of animals, and quantified the compensatory mechanisms utilized by rats affected by different neuropathologies. The study demonstrated how this rung-walking task can be a useful tool for evaluating loss, compensation and recovery of function in many rat models (Metz and Whishaw 2002). These rodent tasks are not aimed at describing the posture of rats, and do not have a component of anticipatory behaviour. These tasks also do not look at how animals cope with perturbations, predictable or otherwise. Extending these lines of research to how predictable perturbations are handled is what this

study has focused on. Rats have been shown to brace against uneven surfaces by facing the drop and attempting to back away from it (Pellis & Pellis, 2005). Knowing this specific and quantifiable behaviour, we expected the drop of the platform to elicit this behaviour in the animals.

The use of rodent models to test postural perturbations is a virtually untapped avenue of research with many advantages; there are a variety of techniques for the study of neural organization available specifically in rat and mouse research. In searching for posture-related paradigms, we noted that the small pool of research on rat posture does not focus on postural behaviour per se. A novel task was therefore developed in order to measure rodent postural anticipatory behavior. This task was designed to bridge research in the fields of both conditioning and posture, defined as anticipatory postural behaviour.

The primary focus of this study was to evaluate whether or not rats could be conditioned to associate a tone (conditioned stimulus; CS) to a perturbation of their base of support (unconditioned stimulus; US) and describe the changes in behaviour, if any, between the naive and trained rat. In order to elicit the unconditioned bracing behaviour previously described in rats (Pellis and Pellis, 2005), the perturbation presented was always at the same speed, amplitude and direction relative to the platform. This was done in order to facilitate conditioning and to provide a reference frame for the observed behaviour. The variables surveyed are well-accepted descriptors of posture in the field of biomechanics (Prieto, Myklebust et al. 1996, Palmieri, Ingersoll et al. 2002): center of pressure (CoP) displacement, CoP excursion, and CoP variability. In order to more robustly describe the animals' behaviour, body orientation, angular displacement, total angular excursion and angular variability were also analyzed.

We hypothesized that trained rats would have changes in CoP and orientation variables post-CS-onset compared to pre-CS, learning the association between the stimulus

tone and the platform drop. We also hypothesized that these differences would not be present in naive animals. This study is our first in describing the behaviour of rats in an anticipatory behaviour paradigm that could lead to new approaches in rodent research.

Materials and Methods

Animals

These experiments were carried out on 6 male Wistar rats, bred in the Concordia University Animal Care Facility. The original breeding colony was from Charles River Laboratories (St-Constant, QC.). The animals were on a 12 hour, reverse light-dark cycle, and were habituated to human contact prior to the beginning of conditioning and to the laboratory and platform before data collection. Animals were weighed every second day of conditioning to monitor body weight. All experimental procedures were approved by the Concordia University Animal Research Ethics Committee. At termination of experiments, the animals were transcardially perfused using 4% paraformaldehyde in 0.1 M phosphate buffer solution, and the brains were extracted.

Apparatus

The training arena consisted of a force plate resting atop a hinged frame (see figure 1A). The frame was powered by 3 electric servomotors with built-in rotation sensors (LEGO® Mindstorms®, LEGO group, DK-7190, Denmark). These motors were controlled with the LEGO NXT micro-computer programmed with a modified version of LabVIEW© (National Instruments Corporation, Austin, TX) included with the Mindstorms® robotics kits. The control parameters included the angular velocity and the total angular displacement of the platform drop. The NXT micro-computer also presented the 1100 Hz stimulus tone and sent time-stamps of all conditioning events to the force plate data acquisition software.

Force Plate

The force plate was designed in collaboration with Loadstar Sensors (Warm Springs, CA, USA). The dimensions of the force plate were 350 mm in the long axis (x-axis) by 255 mm in the short axis (y-axis). It was built with a transparent acrylic sheet and 4 resistive load cells (RAPG1-001M-A, Loadstar Sensors) rated to 4.45 Newtons (454 g) with a sensitivity of 0.02% of maximum load capacity, ≈ 0.0009 N (0.09 g). Force data from the load cells were collected, and the center of pressure (CoP) was calculated in real time by LoadVUE-CG software (Loadstar Sensors), provided with the force plate. The sampling rate of the software was 127 Hz.

Force Plate Testing

Testing of the force plate was done using weights between 20 g and 1000 g on different locations on the force plate, both on ground level and on the platform to simulate the forces to be measured. Subsequently, the CoP data of freely moving rodents were collected, both on the floor and on the platform to check for noise that could arise from a rodent moving on the force plate resting on the platform. A fast Fourier transform analysis was performed on CoP data to check for resonance in the system. The data from the force plate on the platform demonstrated resonance at 13.5 Hz and 27 Hz compared to the data from ground level. For this reason, all CoP data were filtered using a recursive second-order 10 Hz low-pass Butterworth filter.

--- insert Fig. 1 about here ---

Conditioning

Animals were classically conditioned between post-natal days 55-80. The conditioning took place over a period of 15 sessions, where each session consisted of 12 trials. Delay conditioning was used; for each trial, a 2 s stimulus tone (CS) was coupled to the platform perturbation 1 s after the CS-onset (US) with inter-trial intervals lasting between 45 and 120 s (see figure 1B). The perturbation consisted of a 35° angular displacement of the platform (see figure 1C and 1D). A successful trial consisted of an animal remaining on the platform after the platform returned to its original position. Catch sessions were performed at days 16 and 17 of training, which consisted of 13 trials where 6 catch-trials (CS alone with no US) were randomly placed after the first 2 trials throughout the session.

Video

All sessions were filmed from beneath the transparent force plate in order to record the animal's body orientation on the platform. The camera was a Sanyo VPC-CG20 (Sanyo Canada, Woodbridge, Ont.), capturing video at 60 frames per second.

Data Analysis

Center of Pressure Variables

Using the force plate data, the following variables were extracted: CoP position, CoP displacement, CoP total excursion, and CoP variability. All of these variables had both X and Y components which were in the anteroposterior (AP) and mediolateral (ML) directions, respectively. All video data collected were separated into 2 s analysis windows beginning 1 s pre-CS onset and ending 1 s post-CS-onset. Data for each variable was then split into pre-CS

onset and post-CS-onset values for comparison. The force plate data during the platform drop were not used due to artifacts arising from the platform drop.

Displacement was calculated by subtracting initial from final position in each 1 s analysis window, pre- and post-CS-onset.

Total excursion was calculated by summing the absolute value of the differences in position between samples.

Eq 1:

$$Total\ Excursion_x = \sum_{n=1}^{N-1} \left| (x_{n+1} - x_n) \right|$$

Where $[x_1, x_2, \dots, x_n]$ are the observed values of the data points, $\bar{x}$ is the mean value of these observations, and the *N* stands for the size of the sample.

To measure within trial variability, the standard deviation of the CoP was calculated.

Eq 2:

$$CoP_x Variability = \sqrt{\frac{1}{(N-1)} \sum_{n=1}^{N} (x_n - \bar{x})^2}$$

Where $\{x_1, x_2, \dots, x_n\}$ are the observed values of the data points, $\bar{x}$ is the mean value of these observations, and the *N* stands for the size of the sample.

Although root mean squared amplitude (RMS) of CoP is a widely accepted measure of variability in postural control in human studies (Palmieri et al, 2002), a look at the equation for RMS amplitude of CoP sheds light on why this variable is not adequate for this study.

Eq 3:

$$x_{rms} = \sqrt{\frac{1}{n}(x_1^2 + x_2^2 + \ldots + x_n^2)}$$

This equation takes into account the distance of the CoP from the origin. Since animals are free to move anywhere on the platform, two comparable trials, one at the origin and one far from the origin, will have vastly different values for RMS amplitude even though their inter-trial variability are the same. Therefore, the equation for standard deviation was used to compute the trial by trial variability, which we named *CoP variability*.

--- insert Fig. 2 about here ---

Orientation Variables

Video was processed using the video editor VideoPad (NCH Software Inc, Greenwood Village, CO). The stimulus tone waveform was used to crop the video into 1 s pre-CS onset and 1 s post-CS-onset segments. Video was then downsampled to 15 frames per second and processed with custom MATLAB scripts to calculate the orientation of the rat's body. The anatomical landmarks used to calculate the orientation were the urogenital orifice (base) and the midway point between the neck and shoulders (neck) (see figure 2). The angles obtained were calculated using the following:

Eq 4. $$(x, y)_{body} = (x, y)_{neck} - (x, y)_{base}$$

$$\theta = arctan(y_{body}/x_{body})$$

Where (x, y) are the coordinates of the different landmarks, θ is the angle made by the animal's body with respect to the platform.

The MATLAB function *atan2* was used to identify the proper orientation of the rat on a -180° to 180° reference frame (see figure 2 & 3). The *unwrap* function in MATLAB was used on θ to correct for absolute jumps in angles greater than π caused by using the *atan2* function on values close to -180° and 180°. For example, when initial orientation values were less than 180° and final orientation values were more than 180°, the angle data remained continuous and did not jump to negative values. The same held true for initial angles which were more than -180°. Using the video, orientation, angular displacement, total angular excursion and angular variability were calculated in a fashion similar to the CoP variables.

Success rate

Success rate was calculated by dividing each session's successful trials by the total number of trials in the session, and multiplied by 100.

Statistical Analysis

To group the learning stages, the first 4 sessions were pooled together and the last 4 sessions were pooled together, referred to as *naive* and *trained* stages, respectively. The force plate was separated into 4 quadrants; Q1, Q2, Q3 and Q4 (see figure 3).

--- insert Fig. 3 about here ---

All CoP and orientation data were tested for normality using the Shapiro-Wilk test and it was concluded that the data were non-normally distributed. Therefore non-parametric

statistical tests were used to evaluate differences between learning stage (Kruskal-Wallis ANOVA by ranks) and by CS-onset (Wilcoxon Matched Pairs test). Variables were then grouped by quadrant to evaluate quadrant differences (Kruskal-Wallis ANOVA by ranks). Comparisons between quadrants by learning stage were made post-hoc using the Mann-Whitney U test. All variables for catch trials were compared to naive and trained trials in the same way. An α-level was set at $p < 0.05$ for all statistical tests.

Results

All 6 rats remained healthy throughout data collection and completed the study. Body weights increased from 350 – 550 g at the time of perfusion. After removing trials due to errors in data collection (4 trials in naive, 3 trials in trained), the total number of trials analyzed for the naive learning stage was n = 267 (per quadrant, Q1 = 51, Q2 = 52, Q3 = 97, Q4 = 67) and for the trained learning stage was n = 288 (per quadrant, Q1 = 62, Q2 = 35, Q3 = 105, Q4 = 86).

CoP Displacement in the x-axis (CoPDx) (AP)

Fig. 4A shows that in naive rats, there were differences between pre-CS and post-CS-onset in CoPDx values in Q2 and Q4 ($p < 0.05$), demonstrating an effect of CS. In trained rats, there were differences between pre-CS and post-CS-onset values within Q1 and Q2 ($p < 0.05$), with post-CS-onset values being negative signifying a displacement toward the back of the platform. When grouping pre-CS values by quadrant and comparing them across learning stages, there was no overall difference between naive and trained stages, as expected. When looking at post-CS-onset values by quadrant and comparing them across learning stages, there was a difference between naive and trained learning stage values, with trained stage CoPDx

values being more negative, indicating movement toward the back of the platform in Q1 and Q2 ($p < 0.05$) (see Fig. 3 for the platform reference).

CoP Displacement in the y-axis (CoPDy)(ML)

In both the naive and trained stage rats, pre-CS and post-CS-onset values were found to be different within Q2, Q3 and Q4 ($p < 0.05$), demonstrating an effect of CS (see Fig. 4B). When grouping pre-CS values by quadrant and comparing them across learning stages, there was no overall difference between the naive and trained stages, as expected. When grouping post-CS values by quadrant and comparing them across learning stages, there was a change in displacement toward the positive y-axis between naive and trained values in Q2 ($p < 0.05$). For Fig. 4B, negative values in Q1 and Q4 indicate displacements from the outer edge toward the center of the platform, while in Q2 and Q3, positive values indicate displacements from the outer edge toward the center of the platform. Therefore, the large positive CoPDy seen in Q2 indicates the animal moving from the outer edge of the platform toward the center of the platform (see Fig 3 for platform reference).

--- insert Fig. 4 about here ---

CoP Excursion in x-axis (CoPEx)

Fig. 5A shows that for naive stage rats, post-CS-onset values were greater than pre-CS values in Q2, Q3 and Q4 ($p < 0.05$), and that in trained stage rats this difference was observed across all quadrants, demonstrating an effect of CS. When grouping pre-CS values by quadrant and comparing them across learning stages, there were differences between naive and trained

values only in Q2 ($p < 0.05$). When comparing post-CS-onset values by quadrant and across learning stages, trained stage values were significantly greater in Q1, Q2 and Q3 ($p < 0.05$), suggesting an overall increase in the animal's CoP movement post-CS-onset with training.

CoP Excursion in y-axis (CoPEy)

As seen in CoPEx, post-CS-onset CoPEy were greater than pre-CS values in all quadrants for the naive stage rats and Q1, Q2 and Q4 for trained stage rats (see Fig 5B), indicating an effect of CS on excursion. No difference was observed between pre-CS values across learning stages. There was an increase in CoPEy post-CS-onset in Q1, Q2 and Q4, indicating an increase in the animal's CoP movement post-CS-onset with training.

--- insert Fig. 5 about here ---

CoP Variability in x-axis (CoPVx)

For CoPVx, in both naive stage and trained stage rats, there were differences between pre-CS and post-CS-onset values within all quadrants ($p < 0.05$), demonstrating an effect of CS (Fig. 6A). When comparing naive and trained stage rats, pre-CS CoPVx was greater in Q2 ($p < 0.05$), while post-CS CoPVx was greater in Q1, Q2, and Q4. This increase in post-CS-onset CoPVx indicates and overall increase in the animal's CoP movement with training. When considering the post-CS-onset CoPVx values by quadrant and comparing them across learning stages, there were differences between naive and trained values in Q1, Q2 and Q4 ($p < 0.05$), suggesting a combined effect of training and CS on CoP variability in the x-axis for quadrants 1, 2 and 4.

CoP Variability in y-axis (CoPVy)

In naive rats, there were differences between pre-CS and post-CS-onset values within all quadrants ($p < 0.05$), demonstrating an effect of CS (Fig. 6B), with greater CoPVy post-CS. In trained rats, there were differences between pre-CS and post-CS-onset values within all quadrants ($p < 0.05$), demonstrating a similar effect of CS as in naive. When grouping pre-CS values by quadrant and comparing them across learning stages, there were differences between naive and trained values in Q2 ($p < 0.05$). When grouping post-CS-onset values by quadrant and comparing them across learning stages, there were differences between naive and trained values in Q1, Q2 and Q4 ($p < 0.05$), with trained values of CoPVy being greater than naive, suggesting a combined effect of training and CS on CoP variability in the x-axis for quadrants 1, 2 and 4.

--- insert Fig. 6 about here ---

Orientation Variables

Whether in the naive or trained stages rats appeared to have a preferred orientation onto the platform. Post-hoc analysis revealed an effect of quadrant on the orientation of rats, both pre and post-CS-onset (Fig. 7). There were significant differences between the quadrants that made up the right side of the platform (Q1 and Q4) when compared to the quadrants that made up the left side of the platform (Q2 and Q3), with the animals' orientations facing the center of the platform. Although only the orientation data for trained rats are shown, naive data were statistically no different from trained. No differences in orientation were found when comparing Q1 to Q4, or Q2 to Q3 ($p < 0.05$).

--- insert Fig. 7 about here ---

There were no differences in angular displacement between naive and trained rats, pre-CS and post-CS-onset, or between quadrants. For both angular excursion and angular variability, there was a main affect of CS-onset ($p < 0.05$) in all quadrants in both naive and trained rats, except for trained animals in Q3 (Fig. 8). There was no statistical difference between naive and trained values, suggesting no training effect on angular excursion and angular variability.

--- insert Fig. 8 about here ---

Catch Trials

When comparing catch trials to naive trials and to trained trials, there were no significant differences in any variables between pre- and post-CS-onset, quadrant or learning stage. Although there were trends towards differences between trials and conditions, they did not reach significance. This may be due to the low number of catch trials (Q1 n = 9, Q2 = 8, Q3 = 20, Q4 = 18).

Success Rate

There were no differences between the success rate of naive (83.5%) and trained rats (76.7%) ($p = 0.279$). This was expected since the angle at which the platform drops was chosen in such a way to keep the animals motivated and alert, but not enough to entrain learned helplessness.

Discussion

The primary focus of this study was to evaluate whether or not rats could be trained to anticipate a platform perturbation and describe the changes, if any, between the naive and trained rat. In order to elicit the unconditioned bracing behaviour previously described in rats (Pellis and Pellis 2005), the perturbation presented to the animals was always at the same speed, amplitude and direction relative to the platform. This was done in order to facilitate conditioning and to provide a reference frame for the observed behaviour. The variables used are well-accepted descriptors of posture in the field of biomechanics (Prieto et al, 1996; (Palmieri, Ingersoll et al. 2002): CoP displacement, CoP excursion, and CoP variability. In order to more robustly describe the animals' behaviour, body orientation, angular displacement, total angular excursion and angular variability were also analysed. Due to the novelty of this task, care was taken as to not over-extend the interpretation of data. With a clear set of baseline descriptors of expected behaviour in normal rats, future research comparing other rat neurological models to these results may prove useful in an array of research avenues and neurological models.

Anticipatory postural behaviour strategies

Our analysis revealed an effect of training on the CoP variables of the rats, and they learned to use the conditioning tone in order to prepare for the platform drop. All post-CS-onset CoP variables had significant differences in the trained stage compared to the naive stage. The increases in CoP excursion and CoP variability of the trained stage demonstrate that the animals were reacting to the CS by increasing their activity on the platform. Although this provides evidence of a CS-US association, these variables do not describe context-specific changes in behaviour. CoP displacement takes into account the final position of the COP relative to its initial position. In this way, it provides a direction to CoP movement. During the trained stage, the rats had negative displacement values in the x-axis after the CS in Q1 and

Q2. These negative displacements signify a shift in the rats' CoP toward the back of the platform farthest away from the drop, where the animal is most likely to successfully remain on the platform. Similarly, displacement values in the y-axis after the tone show that trained animals in Q2 had more positive values, indicating shifts in the CoP toward the center of the platform, away from the edge. These results indicate that, not only did rats learn the CS-US association over repeated conditioning sessions, but that the rats learned the parameters of the task, anticipated the platform drop and developed a context specific strategy to cope with the perturbation.

Body Orientation does not change with Conditioning

The body orientation results provide complementary information. Although there was no training effect for the orientation variables, there were clear quadrant differences in the body orientation measure. As such, the animals' CoP location in quadrants 1 and 2 demonstrated the greatest range in body orientations regardless of learning stage or CS-onset. Except for Q1, the inter-quartile ranges of body orientation were pointing toward the inner portion of the platform, away from the edges. A general interpretation of the similarities between naive and trained orientation values may be that body orientation is dependent the rats' location on the platform irrespective of training, and that the animals did not want to face the platform edges as a general coping strategy. This may be due to the platform design with no walls around the perimeter. Being oriented away from the edges of the platform, where the animal can fall off, may be a strategy adopted by the animals regardless of training. Additionally, the animals were generally facing the direction that they moved toward after the CS-onset in trained sessions, as is indicated by the results of displacement in the x-axis. Angular excursion was different across quadrants, and although post-CS-onset values in the trained stage were statistically no different than the naive stage, the increases post-CS-onset are indicative of the animals' increased activity on the platform.

Overall, the changes observed between naive and trained rats post-CS-onset: in total angular excursion, CoP excursion and in CoP displacement in quadrants 1 and 2 demonstrate that the animals learned the CS-US association of this task and the parameters of the perturbation. The negative displacement values in the x-axis give a direction to the CoP shift. The quadrant-specific body orientations indicate the animals' preference in facing the internal portions of the platform the increased angular excursion values in trained rats may indicate cumulative body orientation shifts caused by their repeated postural adjustments and paw placements. Our initial hypothesis was that the CoP variables along with the orientation variables would be different post-CS-onset in the trained stage, compared to naive. Although the orientation values did not demonstrate changes associated with training, the CoP values did. We believe this is due to the strategy employed by the animals, which involved a preference to face the interior of the platform and move toward the back of the platform post-CS-onset. Therefore, already facing the proper direction between trials may have been a way to optimize their movement, considering the short time they had to anticipate the platform drop.

The catch trials did not show differences between the naive stage trials or trained stage trials. This could be due to the relatively small number of catch trials compared per quadrant. Success rate was another variable that did not change across learning stages. This was in part expected due to the magnitude of the platform drop; care was taken to present a challenging perturbation each trial but not one that would always elicit falls. This was done to preserve the animals' motivation during sessions and prevent learned helplessness.

A Novel Behavioural Task

While other tasks have focused on time and length measures in order to infer postural performance (Kennard and Woodruff-Pak 2011), our task focuses on the control of the CoP and body orientation parameters. Other tasks have indeed measured rat kinetics using force-

plates, for example to identify the pattern of the CoP and paws at impact from a vertical drop (Welch, Wade et al. 2009), in assessing the effects of a spinal cord injury (Chang, Chang et al. 2010) or in determining body orientation rotations in an actometer paradigm, which can last for long periods of time (Fowler, Birkestrand et al. 2001, Chiesa, Araujo et al. 2006, Fowler, Zarcone et al. 2010). Our approach is more classical in monitoring posture-specific parameters (CoP, body orientation) and in describing the strategies used by the rats to remain stable on the platform (Bouisset and Do 2008). One test, the narrowing beam, gives a measure of length for a balance measure (Russell, Kutchko et al. 2011). We thus believe ours is a more specific test for postural control than the ability to negotiate a narrowing walkway, or to remain on a rotarod apparatus.

Future studies using this paradigm may vary the length of time between the CS-onset and the US presentation to observe different strategies employed by the rats. Along with the CS-US latency, changing the type of conditioning from delay to trace-conditioning may lead to different and interesting results. For example, eyeblink conditioning has looked at how changing these two parameters affect learning, and which brain areas are responsible for the coding of these different modes of conditioning (Weiss et al, 1999; Green and Woodruff-Pak, 2000).

The nature of this task lends itself well to many avenues of research, both in learning and in posture. Testing the performance and anticipatory behaviour differences among models of aging, motor deficit, learning deficits or ataxia to name a few. This task may also serve to compliment the current anticipatory behaviour research and allow for different techniques not available in humans or other animals.

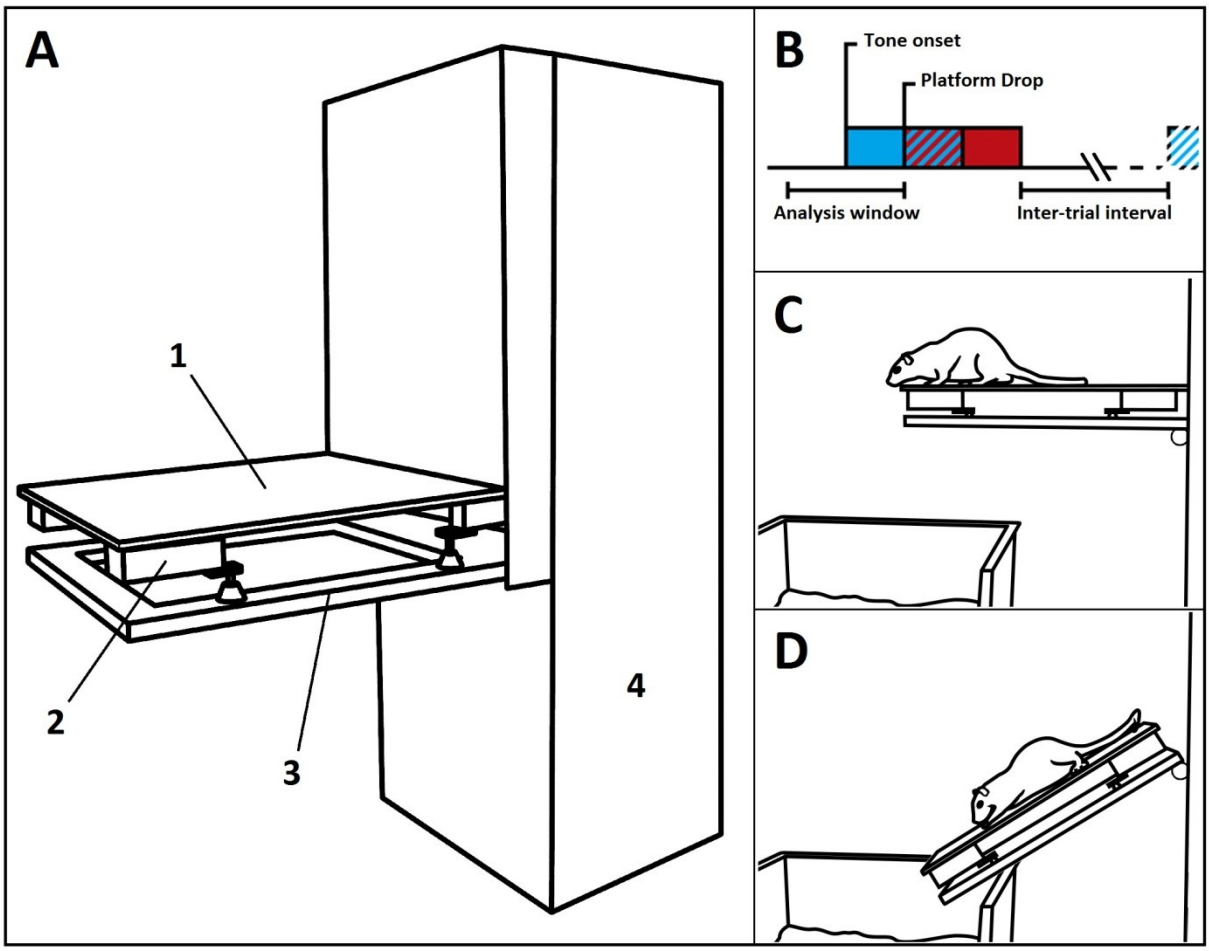

Figure 1. (A) Schematic diagram of the platform. (1) Force plate, (2) resistive load cell, (3) moveable platform, (4) housing for servomotors and NXT-micro computer. (B) Delay conditioning protocol. Platform drop occurs 1 s after CS-onset, followed by a varying inter-trial interval duration. The task: (C) platform in the upright position, (D) platform in the dropped position. Note the box with bedding placed underneath to ensure a safe fall.

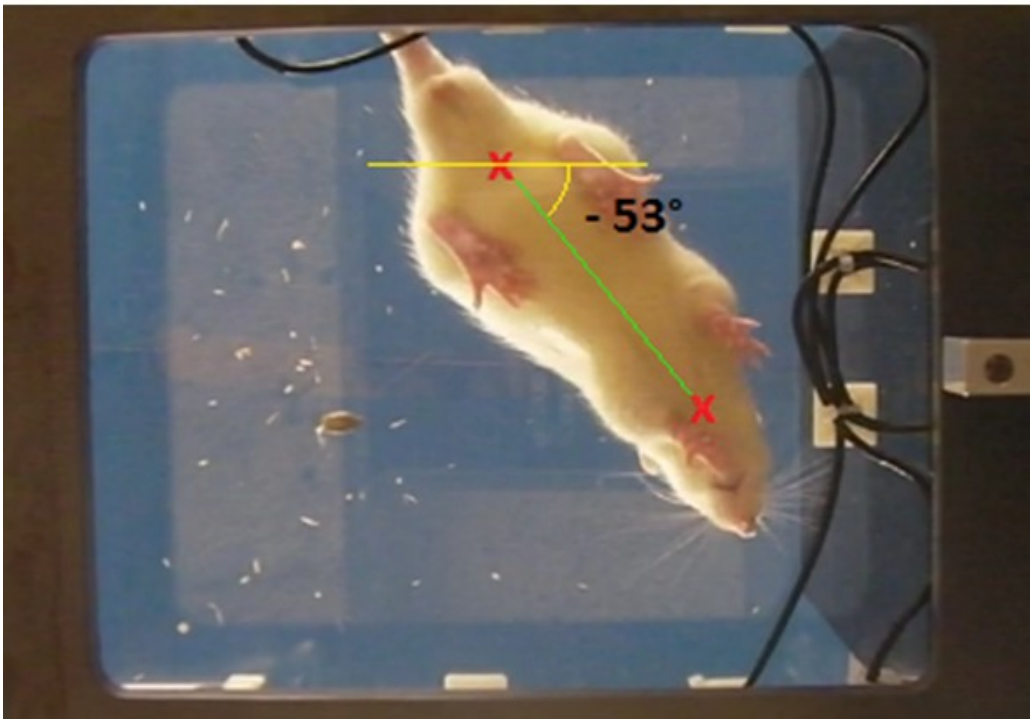

Figure 2. Example of body orientation angle. Shown is a still frame from the video which was filmed from under the platform. The landmarks used to calculate body orientation are marked with an ‘x’. The base of the animal was always considered the origin for the orientation angle (indicated here by a green line).

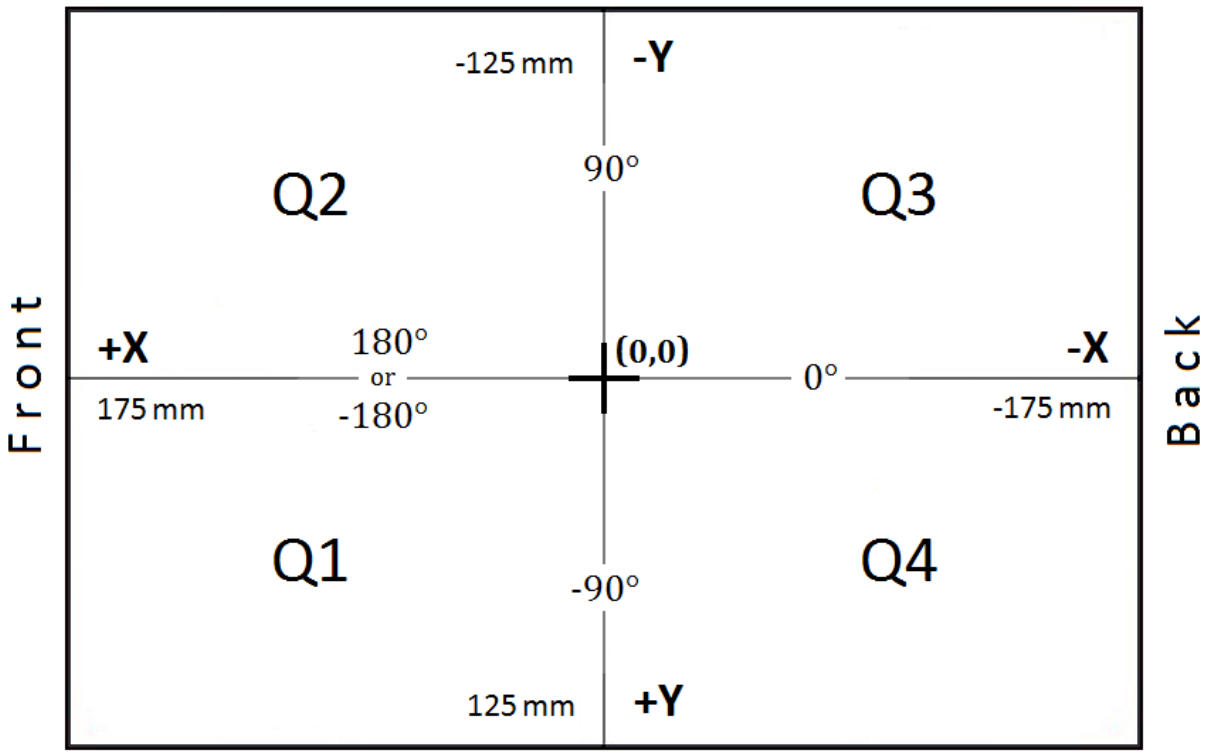

Figure 3. Platform subdivision into quadrants, polar coordinates and relative anlges. The quadrants are separated by the boundaries of the platform about the origin. Of note are the reflected positions of the axes signs and the quadrants when compared to a standard Cartesian coordinate system. The platform spans from 175mm to -175mm in the x-axis and 125mm to -125mm in the y-axis. Additionally pictured is the polar coordinate system used with, 0° pointing toward the back of the platform and -180 or 180 pointing toward the front.

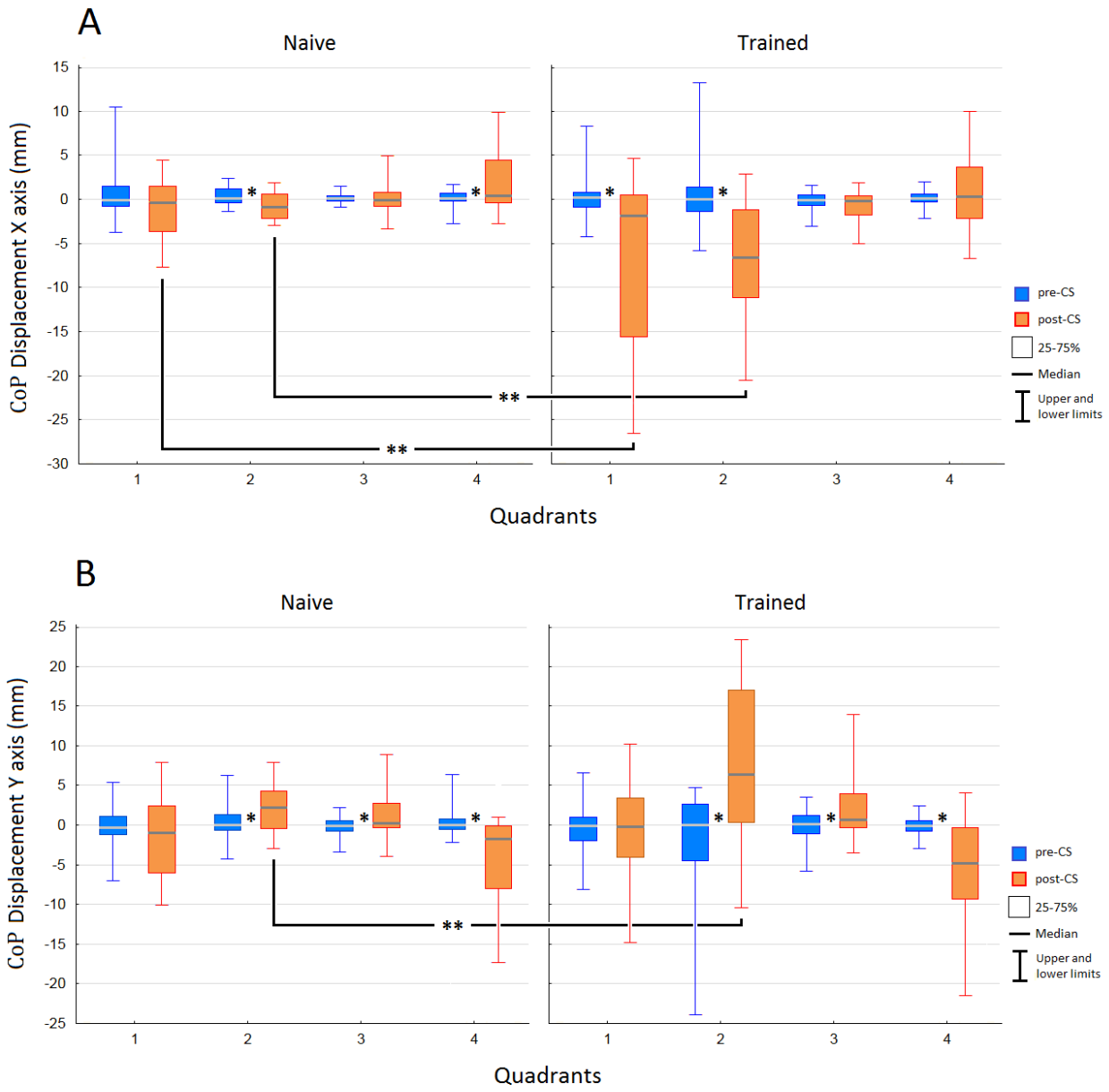

Figure 4. CoP Displacement. (A) Box plot of CoP displacement in the x-axis. Left graph represents the values for naive animals and right graph represents values for the trained animals. Blue and orange boxes represent the pre-CS and post-CS-onset interquartile ranges, respectively. The horizontal line in the box is the median value. Whiskers delimit the 10th and 90th percentiles. (B) Boxplot of CoP displacement in the y-axis, arranged the same as (A). * indentifies pre-CS vs post-CS-onset difference ($p < 0.05$); ** identifies naive vs trained difference ($p < 0.05$).

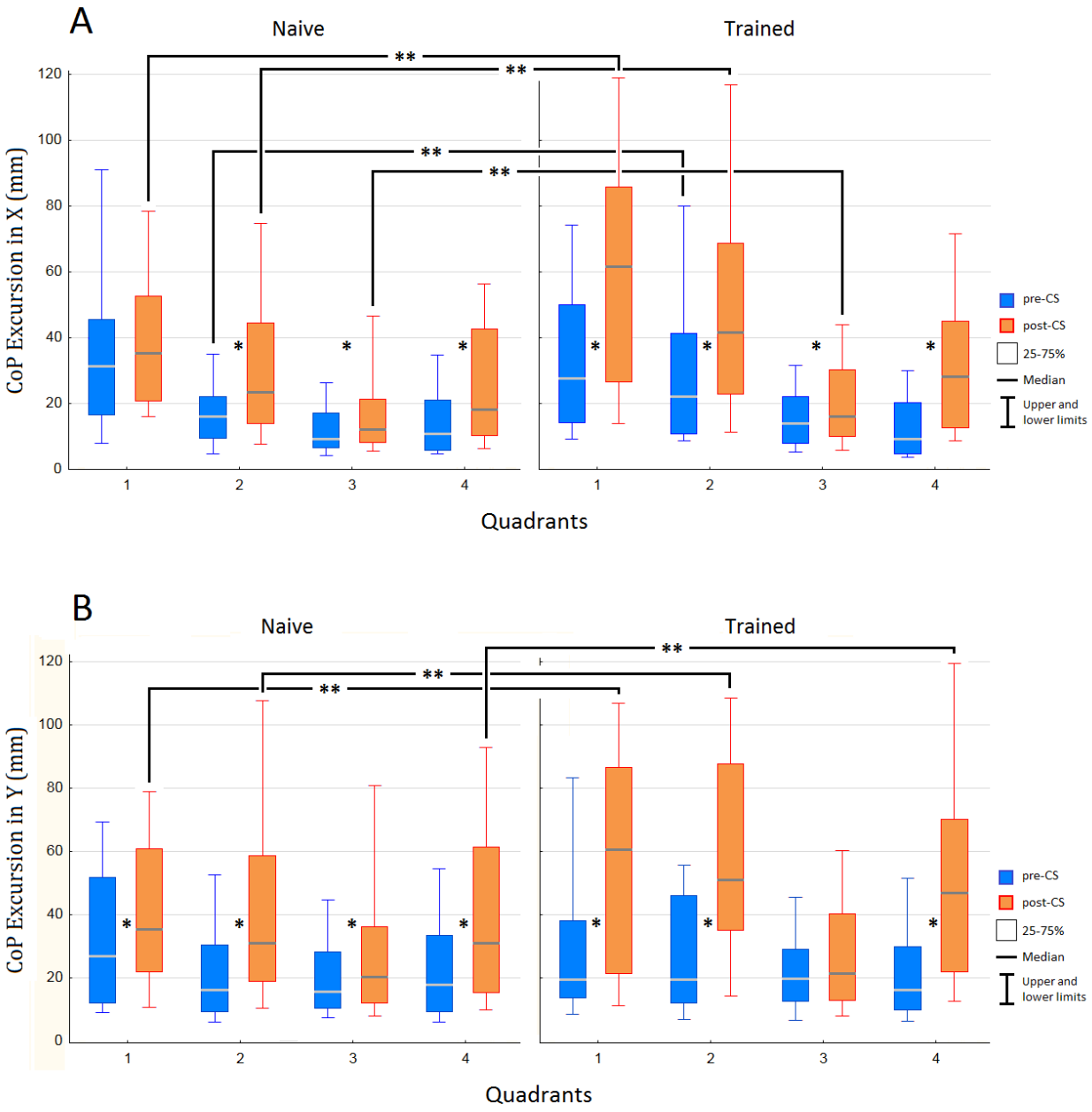

Figure 5. CoP Excursion. (A) CoP excursion in x-axis, (B) CoP excursion in y-axis. Organized like Figure 4. * indentifies pre-CS vs post-CS-onset difference ($p < 0.05$); ** identifies naive vs trained difference ($p < 0.05$).

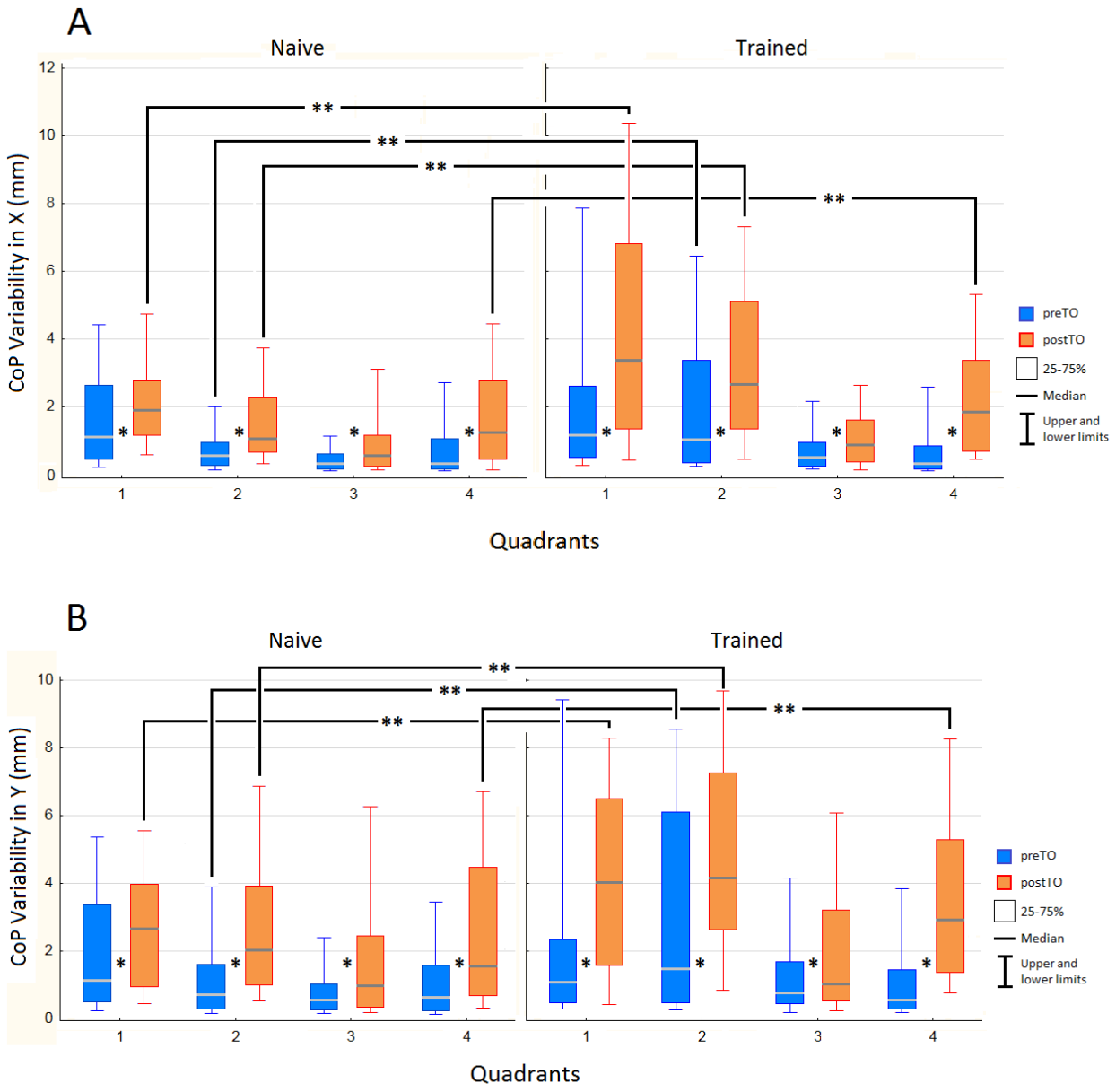

Figure 6. CoP Variability. (A) CoP variability in x-axis, (B) CoP variability in y-axis. Organized like Figure 4. Although not indicated, pre-CS vs post-CS-difference were observed in all quadrants and in both learning stages ($p < 0.05$). ** identifies naive vs trained difference ($p < 0.05$).

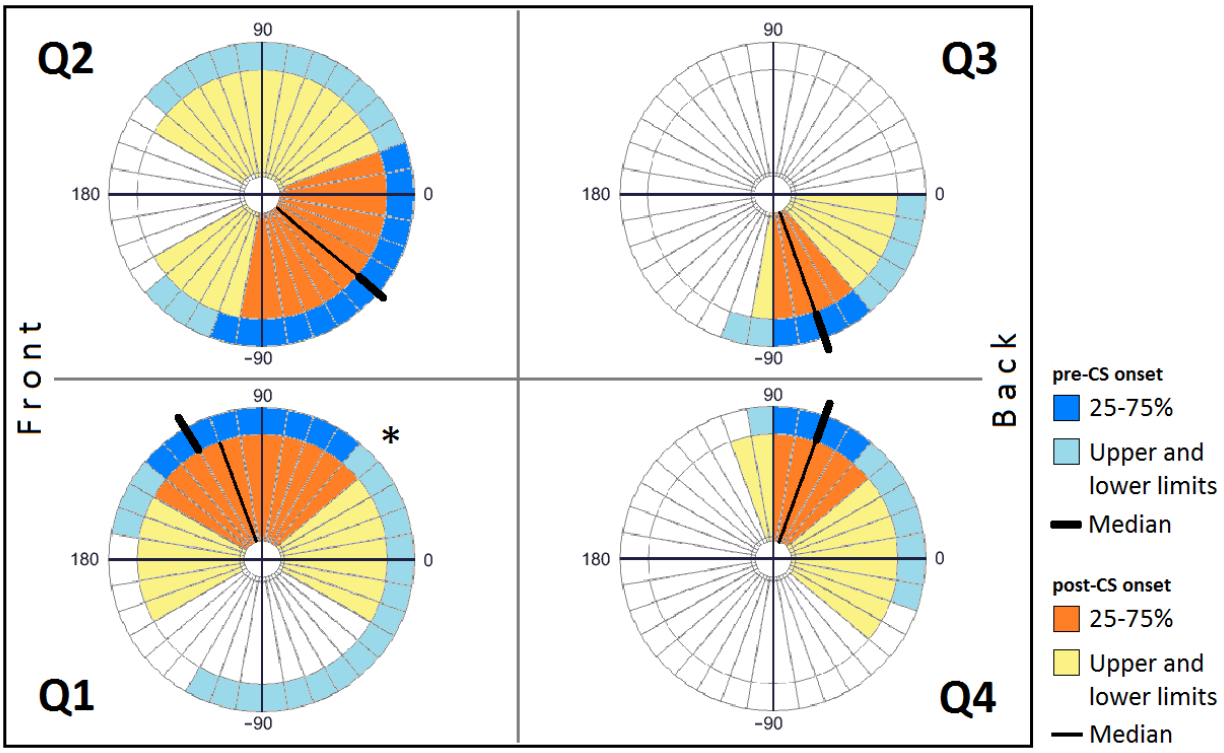

Figure 7. Radial box plot of body orientation data for trained animals, pre- (blue) and post-CS-onset (orange). Data for naive animals were statistically no different from these. The lightly coloured segments (light yellow and light blue) comprise the upper and lower limits, delimited by the 10^{th} and 90^{th} percentiles. There was no effect of training in any quadrant and no effect of CS-onset in Q2, Q3 or Q4. An effect of quadrant can be observed. * indentifies pre-CS vs post-CS-onset difference ($p < 0.05$)

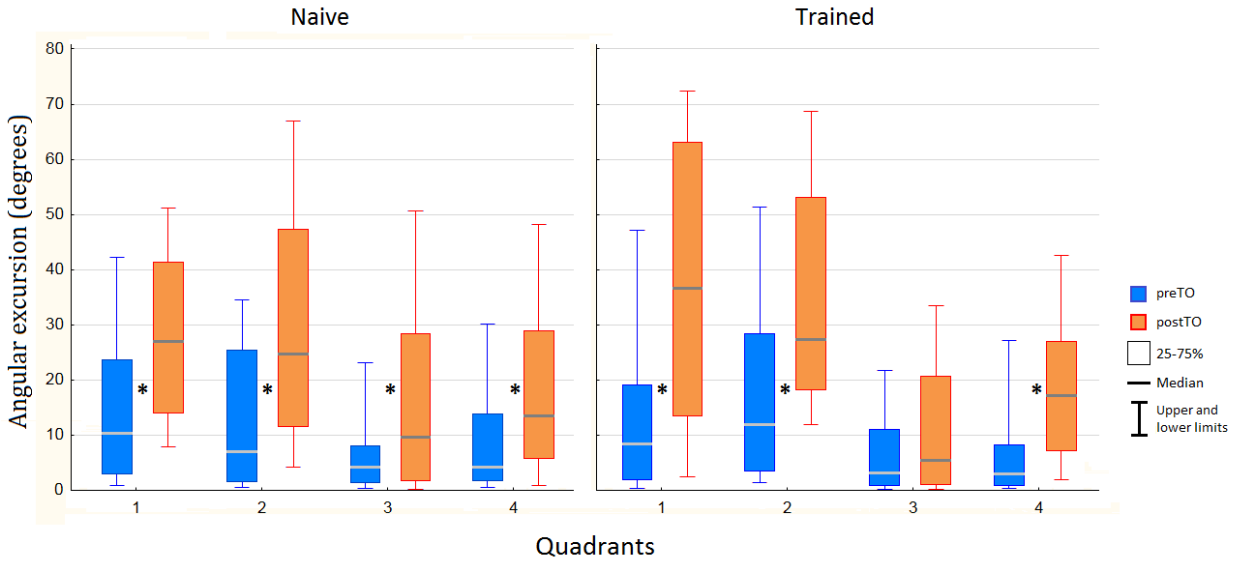

Figure 8. Angular excursion. A quadrant effect and an effect of CS-onset were observed in both naive and trained rats. There was no significant change associated with training. Organized like Figure 4. * pre-CS vs post-CS-onset difference ($p < 0.05$).

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Foetal Alcohol Spectrum Disorder

At the start of this project, one of our objectives was to reproduce the foetal alcohol spectrum disorder (FASD) model in the rat. This model of FASD involves alcohol exposure at a critical period of rapid brain development during post-natal days (PD) 4-9 of rat pups, a period analogous to parts of the human third trimester when many of the same brain areas develop. One of the least stressful methods commonly used to administer alcohol to neonatal pups is by intragastric intubation, i.e. gavage (Goodlett, Peterson et al. 1997).

Our first attempt at recreating this model was with a conservative dosage of 4.5g/kg/day, diluted into baby formula, spread over 2 feedings per day, for 2 consecutive days. This had previously produced moderate morphological changes in brain structures, particularly the cerebellum (Bonthius and West 1991). Each day of gavage consisted of separating the pups from the mother 1 hr prior to feeding (to ensure the pups were not full of milk), and 3 separate gavages. The first two feedings contained the alcohol and the third feeding contained only formula to control for the toxicity of the pups and ensure they did not become malnourished. We also reared a sham group that received the same baby formula but no alcohol. The pups were kept together in small cages between gavages to ensure they kept as much physical contact and to help keep them warm.

After the treatment days, the pups were left to develop normally, weaned and then conditioning began at PD-55. Although there were no visible deficits in behaviour of the animals, the conditioning was still performed in hopes of the task being able to highlight some deficits in the rats, such as lower motor control or learning deficits. A first run of analysis showed that there were no differences between groups in any of the variables (general behaviour, fall rate, CS-US association, etc).

A second group of pups was therefore reared with a higher dose of alcohol (6.6 g/kg/day spread over 2 feedings, for 3 consecutive days), and a sham group with the same number of feedings over the same number of days. This group of alcohol pups did not survive the treatments; they became hypothermic after the first day, lost body weight and had to be euthanized on the third day of treatment. I therefore built a makeshift incubator for the pups, which consisted of pups being in an insulated container surrounded by warm water under a heat lamp. The water and ambient air around the cup was kept around 35° C to mimic the environment they would normally be exposed to in the home cage. This proved successful and this group of pups survived. Again, they were left to develop normally, and conditioning began at PD-55 like all animals.

This group also showed no general behavioural changes: they explored novel cages normally, groomed normally and their body weights were the same. After the conditioning period, pups were transcardially perfused and brains were extracted. A first run of data analysis revealed that significant differences were difficult to establish.. The most prominent and telling variable, displacement in x-axis, showed significant differences in pooled group data over the course of conditioning, ($F_{13, 117} = 2.1282$, $p < 0.05$) but no differences pre-CS and post-CS-onset ($F_{13, 117} = 1.4444$, p = ns) and no differences between groups ($F_{2, 9} = 0.739$, p = ns). The puzzling fact was that the brain and cerebellum weights were different ($F_{2, 13} = 26.23$, $p < 0.05$) ($F_{2, 13} = 13.52$, $p < 0.05$) in the alcohol treated groups compared to sham or controls.

Due to time constraints, a decision needed to be made as to what the focus of work over the following months. We needed to evaluate what the attainable objectives within the master's project were, and concentrate efforts accordingly. Since the main objective of this project was to develop a novel task, we decided to focus our efforts on more rigorously describing the behaviour of animals performing and learning. We therefore shifted our

efforts; we began work on building the video analysis protocol, and decided to separate all data into quadrants.

The reasoning behind the quadrant separation was the nature of the platform setup: the drop always occurred at the front of the platform, there were no edges around the platform and there was a back wall where the animal was furthest away from the drop and therefore the safest. The animal was always free to roam around the platform and therefore had no constraints as to where he was during the CS. This difference in location risk, as it relates to falling off during the perturbation, made us re-evaluate the data.

One of our hypotheses was that the rats would re-orient themselves to brace for the drop. No analysis had been done on the video, and although extensive notes were taken during the conditioning sessions, we wanted objective measures of behaviour to be the basis of our results. We therefore began developing a protocol for analyzing the video. For these reasons, the decision was made to halt work on the neonatal alcohol model and focus on the behaviour task.

Limitations

As with all research, the questions asked and the ways in which they are answered come with limitations. Some of the limitations of our task are inherent to the platform while others were related to technical expertise.

One of the problems was related to the video of the animal's conditioning. Due to the dimensions of the frame holding up the force plate, the animal would sometimes be partially hidden by the frame that held up the transparent force plate. This did not have a profound effect on the collection of body orientation data because landmarks could be estimated fairly easily using the rest of the animal's body. This did, however, affect the ability to use commercially available video analysis software. When we initially began exploring video analysis, we tried 'ANY-maze' software. Although this software was designed to be flexible and adaptable to many different environments, the software was almost never able to reliably track the animal moving on the platform. This led us to develop our own way of extracting orientation data from the video, but it stayed within the confines of a simple rigid-body representation of the animal gross body orientation. If we had more experience or access to more expertise in the field of computer vision, far more information could have been extracted from the video, such as limb position or rearing behaviour.

The force plate itself is was simple in design and could only provide us with ground reaction forces (vertical forces). We could not measure shearing forces in the x- or y-axes and were therefore limited to only using center of pressure as a descriptor. We were also unable to record ground reactions during the platform drop, data which would have complemented and further described the behaviour of the animals.

One of the design issues and eventual improvements we only later realized was the lack of walls around the sides of the platform. In retrospect, clear acrylic walls along the side

of the platform would have been useful for many reasons. The first was that, although the animals rarely voluntarily jumped off the platform, there were some sessions that were prematurely terminated due to this. If the animal jumped off the platform 3 times in a row, the session was terminated. This only happened for 2 sessions out of all of the sessions in the entirety of animals tested (28 animals). Another reason the sidewalls would have been beneficial is because it would have limited the kind of movements the animal could perform. While we never wanted to limit the animal's movement on the platform, the animals sometimes grabbed onto the edge of the platform to help counteract the perturbation. Interestingly though, I was able to note on a few occasions the animals grabbing onto the edge of the platform *at CS-onset*. This is exciting with respect to animal's ability to associate the CS-US and use a variety of strategies to stay on the platform. However, it was not useful for the purpose of this study because there was no way of objectively measuring this behaviour with our setup. Notes were taken during every single trial and behaviour such as this was noted but only verifiable and documented behaviour was used to evaluate learning.

A major setback to this project was the foetal alcohol syndrome aspect. Although I do not think there is necessarily a problem with this model, it is certainly a model that requires a level of experience that we did not have. With the limited time of a master's project and the length of time it takes to mate male and female rats, gestation, weaning and then finally testing, I was only able to apply the treatment a limited number of times. Additionally, since the animals were left to develop normally after the alcohol administration and only began testing around post natal day 45, the animals may have been able to compensate for any deficits the alcohol produced, rendering their behaviour similar to the control rats. Some functional tests could have been performed earlier on in development, to test for the alcohol effectiveness on behaviour at an earlier stage, in order to save time and determine the treatment effectiveness.

Conclusion

The rat is an animal that has not been fully exploited in the field of posture research. The tasks available to use in the rodent are generally functional tests of that evaluate gross performance, locomotion or dexterity. We believe that we have developed a task that can be used to entrain and describe postural adjustment behaviour in rats. Our results suggest that rats can be conditioned to learn the association between a stimulus tone and a platform drop, and that they develop context-specific strategies to cope with perturbations. We believe that this task may be used to bridge some of the research that has been described in the fields of posture and biomechanics. The research literature in humans describing anticipatory postural adjustments provides insight into what might be addressed in animal research. Results demonstrating aftereffects in gait and posture (Reynolds and Bronstein 2003, Bastian 2006, Bhatt and Pai 2009) suggest that the nervous system is able to modify the parameters of gait and posture depending on the context. The electrophysiology experiments done in cats undergoing unexpected postural perturbations provide a framework in understanding which brain areas are responsible for these reactive adjustments (Stapley and Drew 2009). Our task bridges these two separate streams of research and provides a platform on which to build further studies. By utilizing the knowledge of these different experiments, our task can be used to successfully map out the electrophysiological activity of these areas and areas more commonly attributed to learning and balance, and how this activity changes over the course of conditioning.

The synaptic connectivity of different brain areas has been mapped using anatomical tracer techniques. One of these powerful tools for mapping neuronal circuits uses viruses, which transsynaptically infect and replicate neuron populations (McLean, Shipley et al. 1989). Anatomical studies using certain strains of rabies or herpes simplex viruses have been used to

map out the synaptic connectivity of brain areas up to 4 synapses away (McLean, Shipley et al. 1989, Kelly and Strick 2000). For instance, it was recently used to shed light on the connectivity between the basal ganglia and the cerebellum (Hoshi et al., 2005). The nature of the connection between these two brain areas had been a longstanding question that was empirically answered in this study. Most of these viruses infect neurons in a retrograde fashion, but have recently been genetically modified and shown to be able to travel in the anterograde direction in mice and rats (Lo and Anderson 2011, McGovern, Davis-Poynter et al. 2012). Knowing that the pontomedulary reticular formation is one of the areas responsible for reactive postural adjustements, anterograde tracer viruses could be used to reveal the upstream synapses that form a network. This network may be the very quilt on which postural behaviour is knit, beginning at the PMRF as the reactive posture center and leading to higher-function areas where learned anticipatory postural behaviour is crystallized with repeated CS-US exposure. To test this hypothesis, anatomical tracer work would need to be performed; the areas marked by the anterograde virus could then be used as targets for electrophysiology studies in animals undergoing conditioning in our task. The evolution of the neural activity in this network could be tracked over the course of the reactive phase to the perturbation, the acquisition of the motor behaviour and finally the consolidation of the anticipatory postural adjustment. One of the missing elements to performing this sort of study was the correct postural task in the right model animal. We believe our task can fill this role.

From the human studies investigating anticipatory postural adjustments in the broken escalator phenomenon to the electrophysiology studies of cats coping with perturbations, the understanding of which internal processes govern our interactions with the environment is growing. To our knowledge, there is scarce direct research on rodent postural behaviour, and the tests and studies that are available, while viable in discerning nervous system problems, primarily focus on rodent dexterity and locomotion. The studies performed using cats have

answered some interesting questions, but have not focused on anticipation. We believe our task can serve as a platform for future research, bridging the approaches from different domains. This simple twist on already-established paradigms and methodologies may provide insight into the neural substrates governing this simple behaviour.

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