DISSECTING THE DISTINCT CONTRIBUTIONS OF MOTOR MOVEMENTS AND AROUSAL DURING VISUAL BEHAVIORAL PERFORMANCE

by

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Primary Thesis Advisor

The awake brain of an animal is constantly fluctuating between states, causing performance on a given task to be highly variable from one moment to the next. Previous literature demonstrates that optimal performance occurs at an intermediate level of arousal, or wakefulness, following an inverted U-shape pattern. While the effect of arousal on performance has been well studied, there are debates on whether locomotion (walking or running) improves or diminishes the level of engagement on sensory guided tasks. This study aimed to explore the distinct contributions of arousal, whisker movement energy, and speed of locomotion to performance during a visual discrimination task. Results show that the energy of whisker movements and the speed locomotion do not enhance nor diminish performance. Arousal on the other hand correlates with optimal behavior at an intermediate range and decrease with higher states. This result validates previous studies showing intermediate arousal provides optimal performance on a visual task.

Acknowledgements

I would first like to thank Dr David McCormick for taking the time to be my primary thesis advisor and for your continuous support throughout the process. I would also like to thank Postdoctoral Scholar Dennis Nestvogel for being my second reader and mentor every step of the way, teaching me new skills, and most importantly, teaching me patience through it all. Thank you, Dr. Barbara Mossberg, for being my CHC Representative and guiding light for these past 4 years. I would also like to thank Kevin Zumwalt, Daniel Hulsey, and the entire McCormick lab for answering my questions, helping me troubleshoot, and encouraging me throughout this process. Thank you, Clark Honors College, for this opportunity and the amazing staff for their expertise and guidance. Finally, thank you to my peers, Jasper Krehbiel, and Trinity Knight, who proofread, listened to me practice, and sat with me for many hours at coffee shops while I worked.

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Introduction

How sensory information is perceived is highly variable and can change on a moment-to-moment basis. Perception is influenced by state-dependent changes in the brain. For example, at one moment a person may be attentive to a lecture, but the next moment they may find their mind wandering, completely oblivious to the speaker. The brain's ability to attend and respond to sensory information is highly dynamic and regulated by a multitude of factors. For the person attending the lecture, their ability to stay focused could be influenced by factors such as their level of anxiety, motor movements such as tapping a leg, and arousal level. Arousal describes the level of wakefulness in an animal, ranging from drowsiness to extreme vigilance. Understanding the factors that contribute to brain state variability could provide insights into how animals process and perceive the world.

Performance Follows an Inverted U-shaped Relationship with Arousal According to the Yerkes-Dodson Law

The effect of variability in arousal on performance has been studied for over 100 years (Yerkes and Dodson 1908;Cools and D'Esposito 2011;Murphy et al. 2011;McGinley et al. 2015). Research has shown that optimal performance occurs at an intermediate level of arousal in an inverted u-shaped curve. This pattern was first described in 1908 by Robert Yerkes and John Dodson (Yerkes and Dodson 1908). In their classic experiments, Yerkes and Dodson investigated how different levels of arousal induced by varying intensities of electric shocks to the foot of the mice and impacted learning on a visual discrimination task. The mice were tasked to either enter a black box or a white box. If the mice chose the black box they would receive a shock in a range of mild to severe to discourage choosing the black box in the proceeding trial. The researchers discovered an inverted U-shape pattern, now coined the "Yerkes-Dodson curve," in which the mice learned the task fastest at the center between low levels and high levels of stimulation when arousal was intermediate (Figure 1). The findings of this study continue to be a foundation for many psychological and sensory related studies (Teigen 1994;Diamond et al. 2007;McGinley et al. 2015; Yerkes and Dodson 1908;Neske et al. 2019).

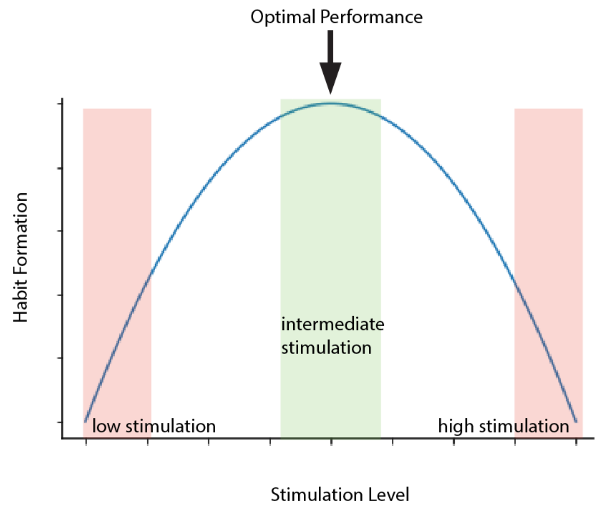

Figure 1. Yerkes-Dodson Inverted U-Curve Describing Optimal Habit Formation.

Yerkes and Dodson found habit formation occurred at an optimal level in a mid-range stimulation level and worsens with increasingly low or high levels of stimulation.

Describing the Anatomy of a Neuron

To understand the mechanisms by which arousal impacts performance during sensory-guided tasks, it is necessary to understand what is happening within the brain. The principal building blocks of the brain are nerve cells-- commonly referred to as neurons. A neuron is a specialized cell which sends and receives signals from neighboring neurons to control an animal's thoughts and functions. A neuron is composed of dendrites, a soma or cell body, an axon, and axon terminals (Figure 2). The cell body is the bulk of the cell which houses the nucleus. The dendrites of the cell receive chemical signals from neighboring neurons. These chemical signals are converted into electric responses that travel through the soma toward the beginning of the axon, a long appendage attached to soma. If the sum of these electric potentials reaches a set threshold, an all-or-nothing response called an action potential is created and the signal is quickly passed through the axon towards the axon terminals. The axon terminals then convert this electrical signal into a chemical signal and pass it on to their neighboring neurons, thereby starting the process in the proceeding neurons. Action potentials can occur in slow oscillations or can occur rapidly in succession. When animals are in a low state of arousal, neurons in the brain typically exhibit slow rhythmic action potentials firing (< 3 Hz). At high arousal, neural activity in the brain (i.e. action potential firing) is highly desynchronized and shows a strong increase in the high frequency range (>30 Hz)

Neurons often do not act individually. State changes are achieved through groups of neurons synchronizing action potentials. For example, imagine the audience at a football game. At first the fans may be talking to their neighbors in separate conversations. Suddenly, a chant starts with a few people, and then the surrounding people join in until everyone in that section shouting in unison. When a change in brain state occurs, such as a shift in focus, neurons in a section of the brain reduce variability and move into synchronized activity.

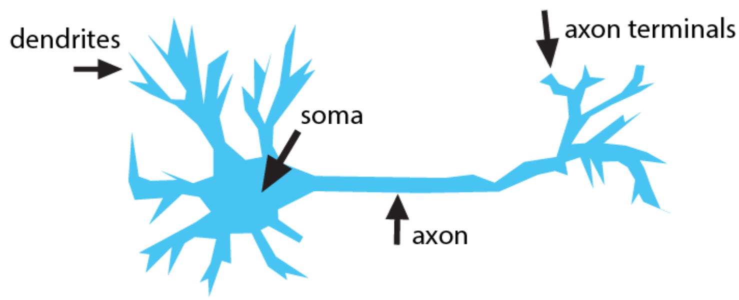

Figure 2. Anatomy of a Neuron.

Neurons communicate with one another at specialized sites called synapses. At synapses, the sending neuron releases chemical messengers into the synaptic cleft-- the space between the axon terminal and the postsynaptic compartment of the receiving neuron. This triggers an electric response in the receiving neuron called a post-synaptic potential, which propagate along the dendrite of the receiving cell and are summed up near the soma at the beginning of the axon. Upon reaching a fixed threshold, a large all-or-nothing response (the action potential) is triggered, and the signal is passed through the axon toward the axon terminals. In the final step, the action potential reaches the synapses of the axon terminals and initiates the release of chemical messengers into the synaptic cleft, thereby activating the next group of downstream neurons.

Arousal Can Be Visualized Through External Behavior

Internal brain states correlate strongly with behavioral states and can in part be inferred by external cues. Studies have demonstrated that non-light related pupil fluctuations are an accurate indication of arousal and match neural dynamics within the brain (Goldwater 1972; Bremner 2001; Loewenfeld 1999; Murphy et al. 2011; Vinck et al. 2015;McGinley et al., 2015). Small pupils are indicative of a quiet wakefulness, whereas large pupils occur during states of high arousal, or excitement. Therefore, changes in pupil diameter can be used as a proxy for changes in brain state without recording from inside the brain.

Whisker movement in rodents, termed whisking, is another indicator for behavioral state that strongly correlates with neural network dynamics (Crochet and Petersen 2006; Poulet and Petersen, 2008; Stringer et al, 2019, Musall et al, 2019, Salkoff et al., 2020, Nestvogel and McCormick, 2022). Mice are nocturnal animals, which use their whiskers to accurately navigate through their environment. Of particular relevance for the present study, mice also display whisking when they are head-fixed and no tactile stimuli are present. In the absence of whisking, slow and large amplitude potential changes are observed in the brain of mice (Crochet and Petersen, 2006; Nestvogel and McCormick 2022). In contrast, active and whisking rodents show higher frequency fluctuations in the brain (Crochet and Petersen 2006; Nestvogel and McCormick, 2022). Whisking has also shown to increase with faster running implying there may be a connection between whisker movement and motor movements such as running (Sofroniew et al. 2014).

It is still unclear whether locomotion (characterized by movement on a wheel) enhances or diminishes performance on sensory-guided tasks. Some studies have associated locomotion in mice with improved response magnitude and higher accuracy during sensory-guided tasks (Polack et al. 2013; Bennett et al. 2013). However other studies have demonstrated a decrease in sensory evoked responses and poorer performance with locomotion (Schneider et al. 2014; Zhou et al. 2014; McGinley et al. 2015).

Performance During Auditory Discrimination Tasks Follow an Inverted U-shaped Relationship With Arousal

A recent investigation of arousal in an auditory discrimination task has observed the Yerkes-Dodson phenomenon in both animal behavior and neural behavior from the auditory cortex (McGinley et al. 2015). A discrimination task requires the subject to decide whether they are receiving a target or not receiving a target. This decision dictates whether the subject performs an action or withholds an action. McGinley et al. designed a task in which mice were trained to lick a spout at the onset of a target sound in order to receive a water reward. The task included a noise stimulus (in which the sound played was not the target sound) and an embedded target stimulus (in which the target was blended into the noise). This last stimulus was crucial for studying arousal because it required the mice to pay close attention in order to determine if there was a target in the midst of the sound. McGinley et al. used locomotion and pupil area to look at arousal on a behavioral and neural level. Their research showed that the mice only walked during high arousal states, and optimal performance occurred in mice with intermediate arousal as predicted by the Yerkes-Dodson law.

Performance on a Visual Discrimination Task is Greatest at an Intermediate Level of Arousal.

Neske et al. conducted a similar study to McGinley et al., instead with a visual discrimination task (Neske et al. 2019). Mice learned to lick when presented with a moving visual target on a monitor with a water reward, and to withhold licking during a distractor stimulus (a visual that is not a target) or a period with no visual (inter-stimulus-interval or ISI). The target was blended into the distractor stimulus in varying degrees of contrast so that in low contrast the target was more difficult to detect among the distractor stimuli. Neske et al. recorded walking and pupil diameter of the mice to determine the correlation between performance, arousal, and movement on a visual discrimination task. Neske et al. found the performance was best at an intermediate level of arousal.

Characterizing Performance On a Visual Discrimination Task.

Performance for the discrimination tasks described above were characterized by four outcomes: hits, misses, false alarms, and correct rejections (Figure 3). If the mouse licked during a target trial, a hit was recorded, and the mouse received a reward. If the mouse did not lick and there was no target stimulus, a correct rejection was recorded. If the mouse licked during a distractor stimulus (with no target embedded) or an ISI, it was characterized as a false alarm. Finally, if a target stimulus was displayed and the mouse did not lick, it was categorized as a miss. To analyze performance, the d-prime was calculated. The d-prime is a statistical measurement which describes how well the subject can discriminate between the noise from target. The d-prime is important because a high number of hits does not necessarily mean a good performance. If a covid test had a 100% success rate in diagnosing real covid cases but it also had a high frequency of false-positives, the test would not be an accurate indication of whether a patient has covid. A higher d-prime indicates the subject can accurately discern between the noise and the target by keeping a high hit rate accompanied with a low false alarm rate.

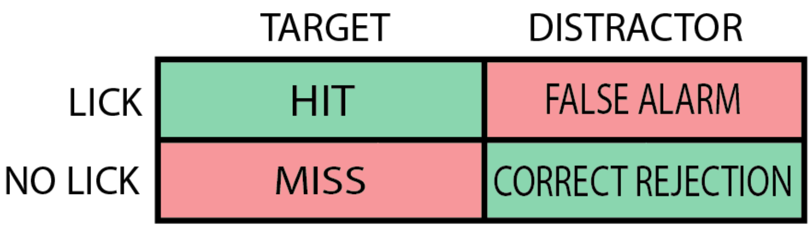

Figure 3. Go-No-Go Discrimination Task

Figure 3 displays the 4 possible outcomes of a Go-No-Go task. A lick during a target stimulus is recorded as a hit. A lick during a non-target stimulus or inter-stimulus-interval (ISI) is recorded as a false alarm. Withholding a lick during a non-target stimulus or ISI is recorded as a correct rejection and withholding a lick during a target is recorded as a miss.

Using a Mouse Model to Study Arousal

The foremost reason why mice are being used in the present study is because our hope in the continuation of this project is to be able to record neural activity inside the brain while the mouse is actively performing the task, requiring a craniotomy and invasive recording techniques. Mice are an ideal model for studying state-dependent variations in the brain due to their ability to learn tasks and quickly respond to sensory stimuli (McCormick et al., 2020). Using a mouse model allows researchers to observe behavior and neural activity simultaneously. Furthermore, extensive knowledge and maintenance of the mouse genome controls variability in behavior due to genetics for posterity in replication experiments (Huberman and Niell 2011).

Project Goals

In the Neske et al. task, the false alarm rate was around 40%. A high false alarm rate could be indicative that the mice did not have mastery over the task. Due to the high false alarm rate, the analysis of the data may not reflect the true behavior. This prompted my host lab to run a similar task with some minor adjustments to try to reduce the false alarm rate. This study investigated the behavioral contributions to arousal in mice during a visual discrimination task by separating pupil, walking, and whisking data relating to performance. In addition, we looked at the speed of locomotion and whisking to determine if the rate of these motor movements influence the quality of performance. We hypothesize these three behaviors play a role in the variability of performance on a visual task.

Methods

Animal Care

All experimental procedures were approved by the University of Oregon Institutional Animal Care and Use committee. Eight mice, both male and female, were used for this experiment. Animals were maintained through the UO animal facility on a 12 hr reversed dark/light cycle. Mice were weighed and then placed on a water restriction regimen (1ml/d). The mice were continually weighed and given a health check to ensure they did not decrease their weight lower than 85% of their pre-restriction baseline weight.

Surgical Procedure

Lightweight steel headposts were fixed on the skull of each mouse (C57/BI6J, age >9 weeks). The mice were anesthetized in an incubation chamber with 5% isoflurane for induction, and then 1.5% isoflurane for the remainder of the procedure. The respiratory activity was monitored, eyes were lubricated, and surgery was performed on a heated pad (37 °C). The hair, skin, and underlying fascia was removed from the surface of the skull. The steel headpost was glued to the skull using dental cement (RelyX Unicem, 3M). After gluing the headpost, the exposed skull was covered with a silicone elastomer (Kwik-Cast, WPI) for protection. During surgery the mice were injected with meloxicam (0.3mg/kg) subcutaneously and allowed to recover on a heating pad until normal behavior returned. For three days after surgery, animals were monitored for signs of infection, discomfort, distress, or pain.

Habituation

After recovery from surgery, the mice were habituated to head fixation and the Styrofoam wheel (20cm diameter, 13cm width). The mice were allowed to freely explore the wheel. This was followed by lifting the mouse to the head fixation post. After a few sessions, the mice was secured to the rig using the headpost and allowed to walk freely on the wheel for a minute. These sessions increased depending on the mouse comfort level until they were up to 30 minutes. To encourage locomotion the mice were gently lifted by the tail to induce an erect posture. Habituation session occurred for a couple days until they were comfortable walking on the wheel.

Task Setup

Fluctuations in arousal were observed using a target-in-noise detection task. Eight mice were fixed in position using a headpost and were allowed to walk freely on a wheel. The first stage was classical conditioning; the mice learned to associate the target stimulus, black bars moving across the screen for 5s, with a free water reward dispensed at the lick spot (Figure 4). Time-outs (no stimulus) of 8 seconds were given when the mouse licked when no target was displayed. In stage 2 the mice were required to initiate the water reward by licking within 100 ms following the onset of the target on the screen. The following stage involved a distractor visual (Gaussian noise; similar to tv static), in which the mice were required to lick during a target and withhold licking during a distractor. A lick during the distractor or during a period with no stimulus (inter-stimulus interval) was punished with an 8 second time out (grey screen). Each trial, either target or noise, was 1.5s long. The proceeding stages increased in difficulty by embedding the target within the noise in varying degrees of contrast. Stage 5 introduced the embedded target (100%, 84%, 68%, 52%, 36%). Stage 6 lowered the contrast making detection more difficult (100%, 70%, 50%, 20%, 5%), leading to the most difficult stage 7 (68%, 32%, 20%, 8%, 2%) (Figure 5). As the mice improved performance on each task, the difficulty increased so that successful detection of the target amidst the background noise required acute attention. All data were analyzed exclusively from stage 7. Visuals were displayed at random, with a probability of 80% for targets. All 4 monitors were gamma corrected.

The task was set up as a go-no-go discrimination task, which is characterized by 4 possible outcomes: hits, misses, false alarms, and correct rejections (Figure 3). Optimal performance was characterized by calculating the d-prime (see statistical methods below). Pupil size, whisker pad movement, and the speed in which the mice walked on the wheel was observed in tandem with the performance.

Licking was monitored using an IR beam and a receptor on two sides of a lick spout. When the mice licked the waterspout, the IR beam would break and a lick would be recorded. The monitor was placed approximately 4 inches from the eye of the mouse. A rotary encoder was placed next to the wheel to record walking speed (Encoder Products CO.; 15T-01SF-2500NV1RPP-F03-S1). Signals from the rotary encoder were translated into units of cm/s using a LabView software. A moving average filter of 20ms was applied to the 25kHz data to smooth the recorded signal. The faces of the mice were illuminated using infrared light along with ambient light coming from one LED light in the box. Pupil size and whisker pad movements were tracked through a program in LabView (Figure 6). In cases where the LabView pupil fitting was erroneous, a program called FaceMap was used to create a better fitting.

Whisker pad motion energy was calculated by a MATLAB script by subtracting the sum of the pixel intensity of each frame from that of the preceding frame within a rectangular border on the face of the mouse. Whisker movement was defined as reaching a threshold of 1 (a.u.) and sustaining above the threshold for at least 2 seconds. Neighboring whisker movement occurring within 1 second or less were grouped together. The mice performed the task in 1.5'x 2' boxes illuminated by a single LED light. The pupil was illuminated by infrared. The head of the mouse was monitored using a Computar camera lens (BN810-43).

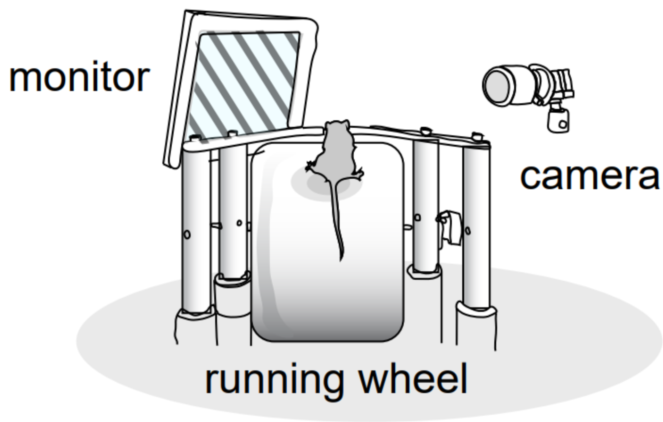

Figure 4. Behavior Set Up

The head of the mouse is attached by securing the headpost to the rig. The mouse is atop a wheel which it can move freely on. The monitor is angled towards the left eye of the mouse while the camera is focused on the right eye. Not pictured: In front of the mouse a waterspout is attached to a syringe that dispenses water if a hit is achieved. In front of the waterspout is a horizontal infrared beam for recording licks. A single LED light is present for ambient light. All equipment is enclosed in a 1.5'x 2' box to exclude unwanted light.

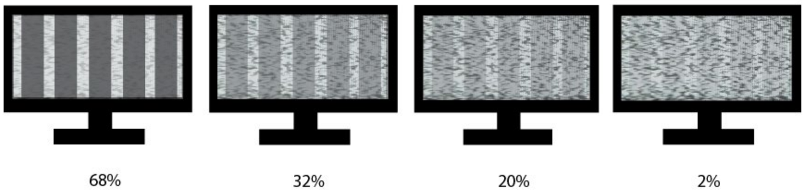

Figure 5. Stage 7 target contrasts.

Targets are blended into distractor visuals in 4 contrasts. The highest contrast (furthest left) is embedded into the distractor visual at 68% opacity, followed by 32% opacity (middle left), 20% (middle right), and 2% (furthest right).

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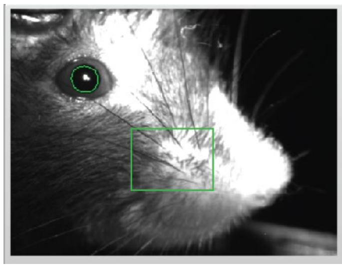


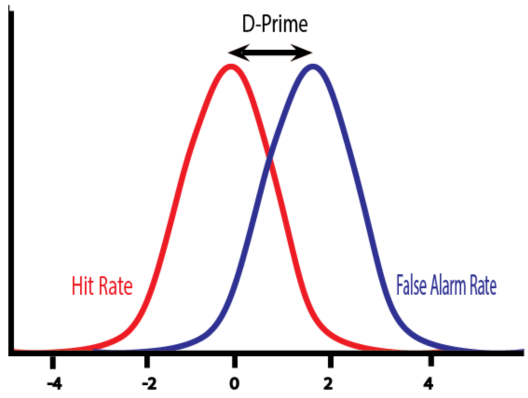
Figure 6. Mouse Pupil and Whisker Fitting.

A LabView script tracks the whisker motion energy (green box) and the pupil change in circumference throughout the session (green circle).

Statistical Methods

The behavioral tasks were run via a script that was written in LabView by Daniel Hulsey. The results were analyzed in Matlab by Dennis Nestvogel using custom written code. To make the data more readable, pupil data were converted into a percentage of the maximum. This allowed all data to fall in a percentage between the minimum (0%), and the maximum value (100%). Figures were also plotted using bins to group the data for ease of visualizing. In addition, a bootstrap confidence interval of the media was applied to the data. A bootstrap confidence interval takes values at random from the dataset to simulate a larger sampling size. All figures simulated a population of 1000. The median of the extrapolated dataset was shaded into the results to indicate the range where 95% of the data falls.

The d-prime is a statistical method which quantifies the ability of a subject to discriminate between a target and a distractor. The d-prime is computed by subtracting the z-transform mean of the false alarm rate (number of false alarms divided by noise trials) from the z-transform mean of the hit rate (number of hits divided by signal trials) (Figure 6). A z-transform is a statistical method used to arrange mean of the data in the center with standard deviations plotted out from the center in what is called a normalized curve. The difference between the mean z-transform hit rate and the z-transform false alarm rate indicates the ability of the subject to successfully detect a target from a distractor. Higher values of d-prime indicate a more precise performance, whereas a lower d-prime value indicates the subject has difficulty discriminating between target and distractor. This method was used to quantify performance on the task.



$$D' = Z\left(\frac{Hits}{Total\ Targets}\right) - Z\left(\frac{False\ Alarms}{Total\ Distractors}\right)$$

Figure 7. D-prime Calculation

The d-prime is calculated by normalizing the hit rate and false alarm rate and then subtracting the two to find the difference between the means. This method quantifies the ability of a subject to discriminate a target from a distractor in a discrimination task.

Results

We hypothesized the level of arousal, whisking, and locomotion would impact performance on a visual discrimination task. In order to investigate this hypothesis, eight mice were trained to lick a waterspout during a target visual in order to receive a water reward and to withhold licking during non-target visuals. The head of the mouse was held in place with a headpost while the rest of the body was free to move on a wheel. The task was characterized by 4 outcomes: hits, misses, correct rejections, or false alarms. Only stage 7 sessions were analyzed, where the target trials were blended into a noise visual in contrasts ranging from 68%, 32%, 20%, 8%, and 5%. A total of 68 sessions from 4 mice were analyzed. Pupil dynamics were recorded to quantify arousal. In addition, walking speed and whisker motion were recorded as indicators of the internal state of the mouse.

Pupil Dynamics, Whisking, and Locomotion are executed in tandem.

This study required a range of arousal levels and behavior so that contributions leading to optimal behavior could be distinguished. Pupil areas for all sessions were compiled into a histogram to determine the range of arousal levels. Arousal varied throughout the sessions from quiet wakefulness, indicated by small pupil area, to bouts of high arousal indicated by a large pupil area (Figure 7). To determine the relationship of arousal, whisker motion, and locomotion on performance, a program created with LabView tracked the circumference of the pupil, whisker motion energy, and walking speed through the duration of the visual discrimination task. Pupil dilations and whisking bouts tightly corresponded (Figure 8). Periods of locomotion also aligned with whisking and pupil dilation but were not required for pupil and whisking activity to occur.

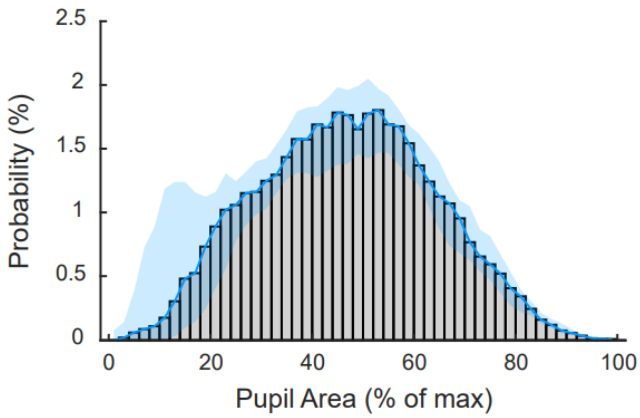


Figure 8: Pupil histogram

The pupil areas across all sessions are plotted above. The data is normalized to the maximum pupil area. Shaded region shows the 95% bootstrap confidence interval applied to the median of the data (see methods).

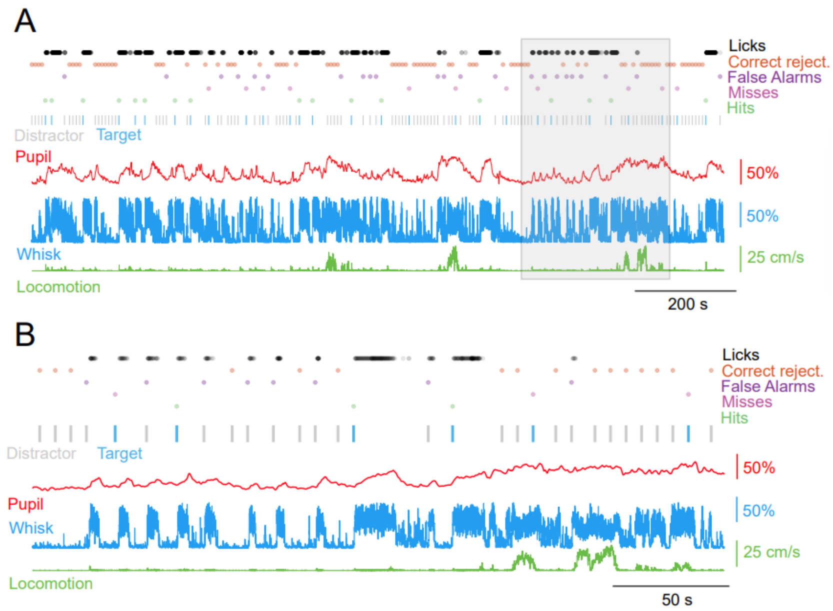


Figure 9. Example Behavioral session for discrimination task.

Part of a session for one mouse is presented above (overview of session shown in figure A, zoomed in section shown in figure B). Every lick is represented by a black dot (top of figure). Below are correct rejections (orange), false alarms (purple), misses (pink) and hits (green). The next layer shows the type of stimulus presented, distractor (grey) or target (blue). In red, the pupil dynamics are shown. 50% of the maximum value is measured on the legend on the right-hand side. The second to last layer (blue) shows periods of whisking. The bottom layer (green) displays locomotion in cm/s.

Hit Rate and Lick Latency by Contrast

To observe different levels of arousal, the targets were embedded into a noise visual in a variety of contrasts ranging from extremely challenging to detect to easily visible (2%, 8%, 20%, 32%, 68%). A typical psychometric task follows an s-shape called a sigmoidal curve, where performance increases as the strength of the stimulus increases. Our results show the hit rate increased as the target contrast increased in an s-shape with an almost perfect hit rate at the highest contrast target (68%), and a 10% hit rate for the lowest contrast target (2%) (Figure 8). A line of best fit shows the results follow a sigmoid curve typical for a psychometric task (Wichmann and Hill 2001). To further explore the distinctions between contrasts, the average lick latency, measured by the onset of the target to the first lick, was plotted for each contrast. Lick latency did not vary widely between contrasts, but higher contrast targets trended towards shorter lick latency periods and low variance, indicating these targets were easier to discriminate (Figure 9).

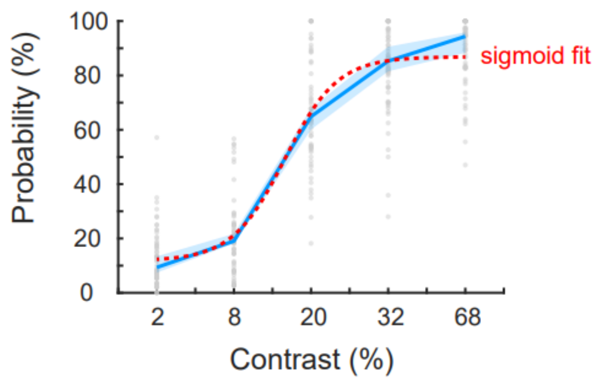


Figure 10. Psychometric curve

Targets were blended into a distractor visual in varying degrees of contrast. The lowest target contrast was 2% visible. The contrasts increased to 8%, 20%, 32%, and 68%. The probability of a hit plotted with the contrast shows a sigmoidal curve (line of best fit shown in red).

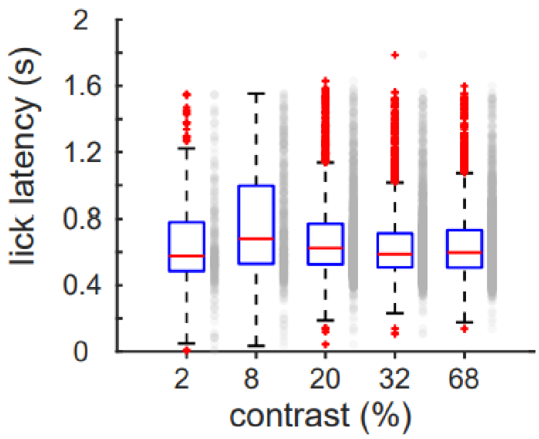


Figure 11: Lick latency as a function of contrast

The seconds between onset of the target and the onset of licking is plotted for each target contrast.

Optimal Performance Achieved by a High Hit Rate, Low False Alarm Rate, and High D-Prime

To determine optimal performance, the hit rate, false alarm rate, and d-prime were calculated across all sessions. The hit rate was calculated by the number hits over the total number of target trials (Figure 10). The hit rate across all sessions averaged at slightly above 50% (A). The false alarm rate, calculated by the number of false alarms over the total number of noise trials averaged at 15% (B). The d-prime measured the performance by calculating the distance between the z-transformation mean hit rate and z-transformation mean false alarm rate. The d-prime for all sessions was 1.2.

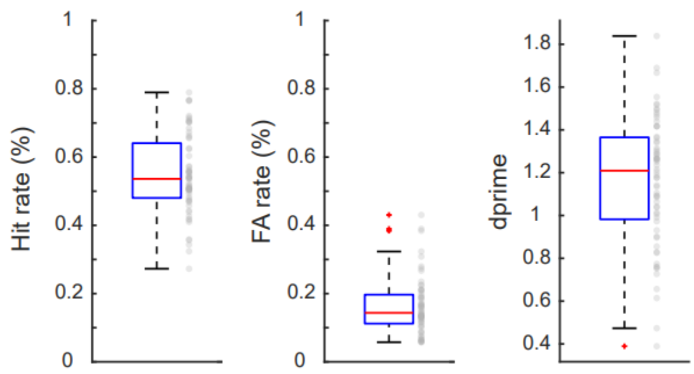


Figure 12. Overall Hit Rate + Overall False Alarm + Overall D-Prime

Average hit rate (A), false alarm rate (B), and d-prime (C) were plotted for all sessions (raw data shown in grey).

Distinct Contributions of Locomotion, Whisking, and Pupil on Hit rate.

Locomotion, whisking, and pupil dynamics were parsed and plotted separately with hit rate. Neither locomotion or whisker motion energy had a significant impact on hit rate (Figure 11A and 11B). Unsurprisingly, pupil area did show a relationship with hit rate (Figure 11C). Hit rate was greatest at low and intermediate pupil ranges, and large pupils were associated with a decrease in hit rate.

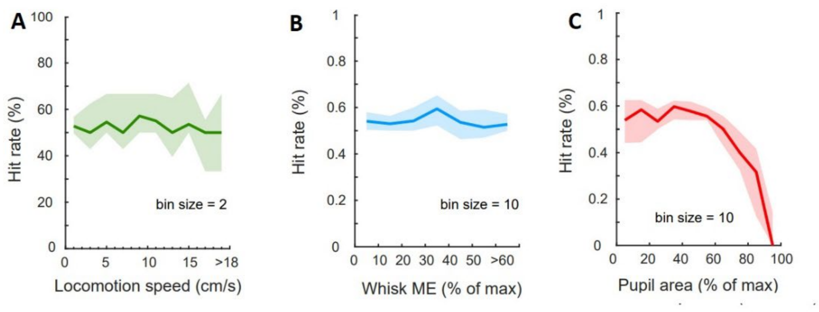


Figure 13: How Locomotion Speed, Whisking, and Arousal Effect Hit Rate

The locomotion speed (A) whisker motion energy (B), and pupil area (C) across all sessions are plotted with hit rate. Locomotion data is grouped into bin sizes of 2. Whisking and pupil data is grouped into bin sizes of 10. Neither locomotion speed or whisker motion energy impact hit rate significantly. Hit rate is highest during low to medium pupil areas and decreases with larger pupils.

Discussion

This project aimed to determine the distinct contributions of arousal and motor movements for optimal performance on a visual discrimination task. Guided by a study designed by Neske et al., the task was modified to decrease the false alarm rate (Neske et al. 2019). We successfully reduced the false alarm rate from 40% to an average of 15%. In addition, we were able to increase engagement to as long as 3-5 hours in some mice, allowing the mice to display several states of wakefulness throughout a session. Our results suggest arousal is a clear indicator of performance in accordance to several previous studies (Goldwater 1972; Bremner 2001; Loewenfeld 1999; Murphy et al. 2011; McGinley et al., 2015). Performance on this task was optimal at low and intermediate levels of arousal and worsened during higher levels of arousal. In addition, our results indicate that locomotion speed has no effect on performance on a visual discrimination task.

False Alarm Rate Was Successfully Lowered.

We hypothesized increasing the duration of the inter-stimulus intervals of the visual discrimination task would result in a lower false alarm rate. The previous task contained short time between stimuli (500ms) which could have led to the high false alarm rate reported by Neske et al. The ISIs were increased from 500ms to 5s. Increasing stimulus interval times allowed for recovery between stimuli. This increased ISI will also be useful for further study during in vivo recordings, by allowing neural activity to return to baseline between trials.

Arousal Did Not Follow a Yerkes-Dodson Curve in 3 out of 4 Mice.

While a range of low and high arousal levels were obtained, only 1 out of the 4 mice displayed a full inverted U-shape relationship between arousal and performance. Several studies have shown that arousal and performance typically follow the Yerkes-Dodson curve (McGinley et al. 2015; Goldwater 1972; Murphy et al. 2011; McCormick et al. 2020; Cools and D'Esposito 2011; Neske et al. 2019; Teigen 1994; Bremner 2001). The discrepancy between the expected result and the 3 mice who did not show this trend could be a result of several factors. First, the pupil fitting in the LabView script was not always able to capture the dynamics accurately. We attempted to solve this problem by using a post-pupil fitting program created in Python (script provided by Daniel Hulsey). The program was able to fix a few of the sessions, but there were still a handful of sessions that neither program was able to correctly map. It is possible these programs were not able to pick up on small pupil ranges and therefore the data for low arousal levels could be skewed from only having a few data points. Future research may be able to solve this issue by using machine learning to identify pupils (Mathis et al. 2018).

Another contributor to the discrepancy could be mastery of the task. Mice are trained for several weeks on the task. Each mouse is evaluated on performance before advancing to the next stage. It is possible that by the time some mice made it to stage 7 they were experts at the task. If the mouse was not challenged sufficiently, their hit rate could be high during lower arousal levels. For example, a person who brushes their teeth every day could perform this task when they are very sleepy because it does not require as much focus. In future research, it may be useful to create fewer stages to decrease the likelihood of mastery.

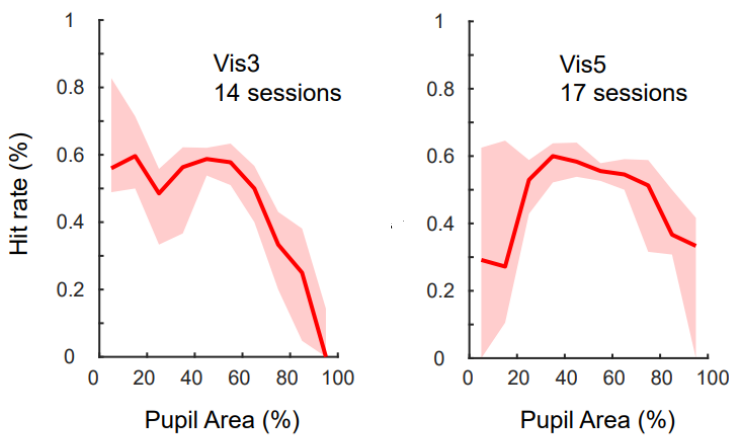


Figure 14. Examples of Variability of Performance and Arousal between Mice

The figure above shows two examples of the variability between pupil dynamics in individual mice. The mouse labeled Vis3 (left) displayed a high hit rate during low and intermediate pupil areas and a large decrease in performance for higher pupil areas. The inverted U-shape is displayed for the mouse labeled Vis5 (right) with a less dramatic variance in performance overall.

Locomotion and Whisking Are Not a Clear Indicator of Performance.

Previous studies have shown that locomotion tends to co-occur with high arousal states and decrease performance during sensory guided tasks (McGinley et al. 2015; Neske et al. 2019). However other studies have shown locomotion improves performance on visual detection tasks (Bennett et al. 2013). The dissonance between these results prompted us to investigate whether the speed of locomotion plays a role enhancing or diminishing performance during a visual task. Our results showed there was no clear indication that the speed of locomotion had any effect on the ability of the mouse to perform. Similarly, while whisking was present during all levels of engagement in the task, the energy of whisker motion did not play a role in performance. These results indicate the action of such motor movements coincide with arousal in an all-or-nothing fashion. The speed of locomotion or whisking do not impact performance.

Pitfalls and Future Research

There are both benefits and disadvantages to using a go-no-go discrimination task. On one hand, it is easy to record and collect data because there are only 4 possible outcomes. The disadvantage of this study design is that it can be difficult to determine when a mouse is purposefully not licking to indicate recognition of a distractor, or if it is not licking because it is distracted/disengaged from the task. A consideration for future studies might be to create a two-alternative forced choice task that requires an action from the mouse for a target as well as for a distractor. For example, instead of having one waterspout that dispenses water for a hit, there could be one waterspout on the left side that dispenses for a hit and one on the right side for a correct rejection. This design would also make the performance clearer during more difficult contrasts. As shown in Figure 10, lick latency at the 2% contrast were on average shorter than the 8% contrast. It is improbable that the mice were faster at detecting the 2% contrast target than the 8%, which means it likely that some of the hits recorded for this contrast were non-intentional licks. A two-alternative forced choice task would give us a better idea of whether the mouse was identifying the target or just giving false alarms. The difficulty with two-alternative forced choice tasks is it requires a lot more training for the mice and it makes quantifying the performance more complex.

If I were given the chance to improve this study, I would create a criterion for disengagement. The mice performed long sessions, sometimes over 3 hours. Disengagement was more easily detectable in some mice compared to others. If performance slowly decayed rather than completely stopped, it was difficult to determine if a mouse was temporarily checking out or done behaving. Due to this some sessions would start with a high d-prime but end up with a much lower value due to worsening performance at the end. Setting a criterion for disengagement that excludes the slow disengagement period of the session may provide results that reflect a more accurate performance.

Another aspect of the study I would adjust is the contrasts included in stage 7. The mice were poor performers at 8% and 2% contrast targets, and very good performers at 20% contrasts and above. The ideal contrast for this study would be difficult enough to only be detected during optimal arousal states. As shown in our psychometric curve (Figure 9), this ideal contrast exists between 8% and 20%. A contrast between these values might be a better indicator of what distinct behaviors lead to detection of a target, and what behaviors cause the mouse to not detect the target.

Finally, if given more time I would be interested to see the internal behavior in the primary visual cortex (V1) and the dorsal lateral geniculate nucleus (dLGN) during a session. The dLGN is the bridge between the retina and the visual cortex. Visual input is sorted in the dLGN and sent to the primary visual cortex to be interpreted and perceived. Previous studies have shown high arousal and locomotion enhance sensory responses in V1 (Niell and Stryker 2010; Bennett et al. 2013; Polack et al. 2013; Neske et al. 2019). By inserting electrodes in these regions and recording neural evoked responses during the task, we would be able to view state changes both externally and internally from awake behaving mice. This could provide insight into how whisking and locomotion affect neural dynamics in ways which cannot be observed externally.

Summary

In this project we were able to modify a previous study to reduce the false alarm rate and observe longer sessions. Our results add to the growing literature demonstrating optimal performance on sensory guided tasks at intermediate levels of arousal. In addition, our results indicate that the speed/motion energy of motor movements such as locomotion and whisking do not impact performance on a visual task. This study offers groundwork for future research on the contributions of arousal and behavior to the variability of state changes within the awake brain.

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