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PERCEPTION OF TACTILE VIBRATIONS AND A PUTATIVE NEURONAL CODE

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Abstract

We devised a delayed comparison task, appropriate for human and rats, in which subjects discriminate between pairs of vibration delivered either to their whiskers, in rats, or fingertips, in humans, with a delay inserted between the two stimuli. Stimuli were composed of a random time series of velocity values ("noise") taken from a Gaussian distribution with 0 mean and standard deviation referred to as σ1 for the first stimulus and σ2 for the second stimulus. The subject must select a response depending on the two vibrations' relative standard deviations, σ1>σ2 or σ1<σ2. In the standard condition, the base and comparison stimuli both had duration of 400 ms and they were separated by a 800 ms pause. In this condition, humans had better performance than did rats on average, yet the best rats were better than the worst humans. To learn how signals are integrated over time, we varied the duration of the second stimulus. In rats, the performance was progressively improved when the comparison stimulus duration increased from 200 to 400 and then to 600 ms. In humans, the effect of comparison stimulus duration was different: an increase in duration did not improve their performance but biased their choice. Stimuli of longer duration were perceived as having a larger value of σ.

We employed a novel psychophysical reverse correlation method to find out which kinematic features of the stochastic stimulus influenced the choices of the subjects. This analysis revealed that rats rely principally on features related to velocity and speed values normalized by stimulus duration – that is, the rate of velocity and speed features per unit time. In contrast, while human subjects used velocity- and speed-related features, they tended to be influenced by the summated values of those features over time. The summation strategy in humans *versus* the rate strategy in rats accounts for both (i) the lack of improvement in humans for greater stimulus durations and (ii) the bias by which they judged longer stimuli as having a greater value of σ.

Next, we focused on the capacity of rats to accomplish a task of parametric working memory, a capacity until now not found in rodents. For delays between the base and comparison stimuli of up to 6-10 seconds, humans and rats showed similar performance. However when the difference in σ was small, the rats' performance began to decay over long inter-stimulus delays more markedly than did the humans' performance.

The next chapter reports the analyses of the activity of barrel cortex neurons during the vibration comparison task. 35% of sampled neuron clusters showed a significant change in firing rate as σ varied, and the change was positive in every case – the slope of firing rate versus σ was positive. We used methods related to signal detection theory to estimate the behavioral performance that could be supported by single neuron clusters and found that the resulting "neurometric" curve was much less steep performance than the psychometric curve (the performance of the whole rat). This led to the notion that stimuli are encoded by larger populations. A general linear model (GLM) that combined multiple simultaneously recorded

clusters performed much better than single clusters and began to approach animal performance. We conclude that a potential code for the stimulus is the variation in firing rate according to σ, distributed across large populations.In conclusion, this thesis characterizes the perceptual capacities of humans and rats in a novel working memory task. Both humans and rats can extract the statistical structure of a “noisy” tactile vibration, but seem to integrate signals by different operations. A major finding is that rats are endowed with a capacity to hold stimulus parameters in working memory with a proficiency that, until now, could be ascribed only to primates. The statistical properties of the stimulus appear to be encoded by a distributed population.

1. Introduction

1.1 Overview

This thesis reports experiments in which we trained rats to compare two successive vibrations applied to their whiskers. The overall design of the project is based on measurements of three variables – (i) sensory stimuli, precisely controlled and quantified by the experimenter, (ii) neuronal activity, and (iii) the rat's percept on each trial, as revealed by its behavioral choice. From these variables, the following three relationships emerge. First, by *psychophysical* methods, we can can measure the relationship between the stimuli and the rat's percept, and learn about how the subject experiences stimuli according to a scale along some physical dimension. Additionally we can perform reverse correlation between behavior and the physical parameters of stimuli to learn about strategy and physical parameters that subjects rely on the most. Second, by measuring the relationship between the stimuli and neuronal activity, we aim to learn about *sensory coding* – how the brain converts physical events into the neuronal language of spike trains. Third, by measuring the relationship between neuronal activity and the rat's percept, we aim to learn about *decoding* and *decision making* – how the neuronal representation of a stimulus is transformed into a behavioral choice (Figure 1.1A). The results on rat behavior constitute an extensive body of data while the neuronal recordings were initiated later and constitute a smaller body of data.

A second set of experiments involved human subjects. This study used the same mechanical vibrations as used with rats, but the stimuli were applied to the finger tip. Without measures of neuronal activity, only one of the relationships described above could be elucidated, the connection between stimulus properties and the subject's percept (Figure 1.1B). The psychophysical results can be valuable nonetheless, for they give us insights into how the neuronal basis of perceptual functions might be different or similar in rats and human. Moreover by using some advanced psychophysical methods we can aim to unveil the subjects' strategies in the tactile task (Neri and Levi, 2006).

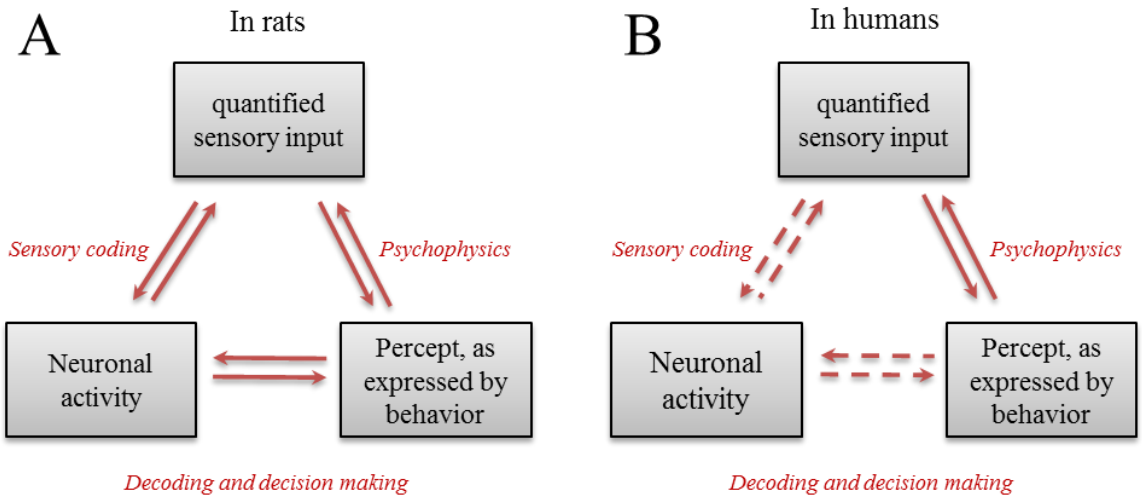


Figure 1.1. Overall structure of experiments. The research strategy is based upon correlations between measurable experimental variables. (A) In rats, three variables, physical stimuli, neuronal activity and animal choice, can be connected. (B) However, in human neuronal activity cannot be measured directly and only informed guesses and inferences (indicated by dashed lines) can be made. This structure of the project allows us to look for differences and commonalities in perceptual function of rats and humans.

1.2 The whisker sensory system

Mice and rats were adopted as laboratory animals for reasons having little to do with integrative neuroscience, but we now know that they possess "expert" sensory processing systems. In nature, they are active in dark environments and have poor vision; their survival depends on the sense of touch. They use their whiskers to recognize the positions of floors, walls and objects, particularly in dark surroundings. A classic study in 1912 illustrated that a rat's ability to navigate through a raised labyrinth depends on the use of its whiskers (Vincent 1912). Modern research has shown that whisker touch (along with olfaction) represents a major channel through which rodents collect information from the nearby environment (Diamond, von Heimendahl et al. 2008).

In this section, we outline the organization of the whisker sensory pathways and in the next section, **Active sensing,** we introduce functional considerations.

Whisker and follicle

Inspection of the rat's snout reveals the grid-like layout of about 35 long and thick facial hairs known as vibrissae or whiskers (Figure 1.2A). These constitute an array of highly sensitive detectors that project outwards and forwards from the snout to generate and collect tactile information. The sensory pathway passes through the brain stem and thalamus before reaching the barrels of the primary somatosensory cortex, SI (Figure 1.2B).

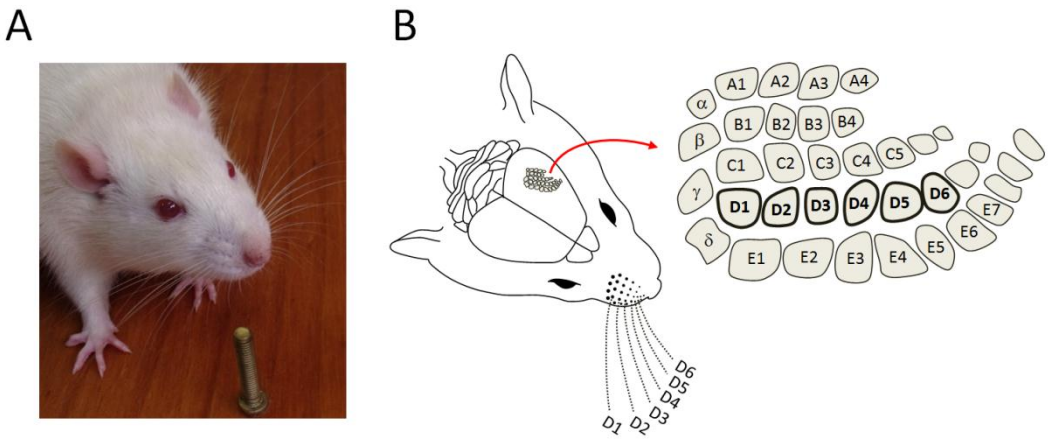


Figure 1.2. Whiskers and barrels. (A) Close-up of a Wistar rat as it explores objects using its whiskers. (B) Arrangement of the barrels in the left somatosensory cortex of a rat, with each barrel labeled by its corresponding whisker. Whiskers of the D row are shown full length with their corresponding barrels highlighted in cortical map. Figure adapted from (Diamond and Arabzadeh 2012).

Whiskers are hollow, tapered shafts; the cuticle of the whisker consists of flat scales, overlapping like roofing slates (Williams and Kramer 2010; Voges, Carl et al. 2012). Another characteristic of whiskers that differentiates them from ordinary hairs is the large follicle, densely populated with various types of nerve endings (Ebara, Kumamoto et al. 2002; Diamond 2010). Whisker motion transmits mechanical energy to the follicle (Birdwell, Solomon et al. 2007) which is transduced into trains of action potentials by sensory receptors—the terminals of trigeminal ganglion cells. Follicles are arranged in five horizontal rows (A to E). There are 4 follicles in rows A and B, and 9 to 12 follicles in rows C, D and E. All follicles of row A and B and the first 7-8 follicles of rows C to E contain big whiskers also known as macrovibrissae (Brecht, Preilowski et al. 1997). Each whisker is identified by a unique letter-number combination corresponding to its row and arc (e.g. row D, arc 2, or D2).The vibrissa follicle (Figure 1.3) is populated by receptors with assorted morphologies and locations (Rice, Mance et al. 1986; Ebara, Kumamoto et al. 2002). Among the most prominent are Merkel endings. Other populations include lanceloate endings, which are a form of free nerve ending. The relations between the morphology and location of a receptor and detailed neuronal response properties remain unknown; to date, ganglion cell responses have been studied without knowledge of the cell's terminal structure. It is known that many neurons in the trigeminal ganglion are sensitive to features of whisker motion, such as velocity and acceleration (Shoykhet, Doherty et al. 2000; Jones, Lee et al. 2004; Arabzadeh, Zorzin et al. 2005). Other ganglion cells are slowly adapting and appear suited to encode whisker position (Lichtenstein, Carvell et al. 1990; Shoykhet, Doherty et al. 2000).

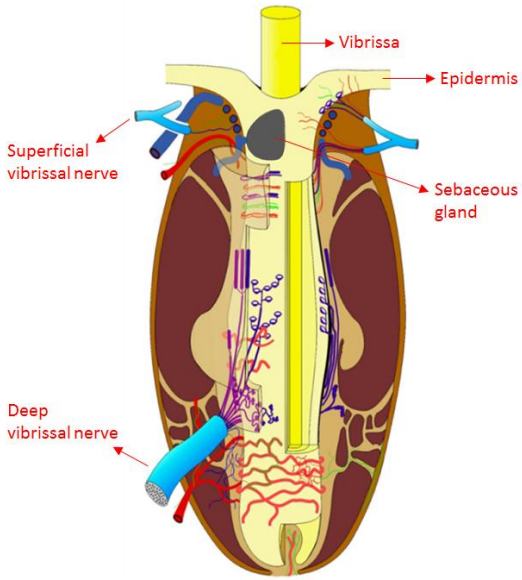


Figure 1.3. Schematic view of the whisker follicle of a rat or mouse. Nerve terminations enter through the superficial vibrissal nerve and the deep vibrissal nerve to occupy different locations within the follicle, and their positioning is likely to be closely related to type of hair movement that excites them (vibration, bending, pulling, etc.). Picture courtesy of Frank Rice, adapted from (Nicholls, Martin et al. 2011).

Recently, Mitchinson and colleagues (Mitchinson, Gurney et al. 2004; Mitchinson, Arabzadeh et al. 2008), followed by Lottem and Azouz (Lottem and Azouz 2011), proposed mechanical and mathematical models of transduction in the whisker follicle. The most recent of these models is notable because it uses a single parameter that determines the time course of the interaction between whisker and receptor. In spite of the complex anatomical structure and the variety of receptor types that exist within the vibrissa follicle, this model successfully predicted the responses of sensory receptor neurons to a number of complex tactile stimuli (Lottem and Azouz 2011).

The ascending pathway

Trigeminal ganglion cells emit a process that divides near the cell body to form a peripheral branch and a central branch. The sensory receptor endings described above are the terminals of the peripheral branch (see details in (Nicholls, Martin et al. 2011)). About 200 ganglion cells innervate each whisker's follicle (Clarke and Bowsher 1962; Dörfl 1985). The central branch enters the brain stem to form synapses in the trigeminal nuclei (Torvik 1956; Clarke and Bowsher 1962). The trigeminal nuclei convey afferent vibrissal information to the thalamus via parallel pathways which then continue to the somatosensory cortex (Deschênes, Timofeeva et al. 2005). Somatosensory cortex, defined as the area receiving direct input from the ascending somatosensory pathway, consists of a primary field (SI) and a secondary field (SII).

The primary field, SI, has been studied intensively in rats and mice. In this area, macrovibrissae have a distinct representation. Both histological (Woolsey and Van der Loos 1970) and electrophysiological (Welker and Woolsey 1974) studies demonstrated a one-to-one correspondence between macrovibrissae and *barrels* – distinct clusters of neurons in SI. Hence, the whisker-receiving area of SI is often called *barrel cortex*. In addition to the wealth of knowledge provided to developmental neurobiology (Andres and Van der Loos 1985), the elegant topography of the sensory pathway offers a great convenience to behavioral neurophysiology: by simultaneous recording of barrel cortical activity and video-monitoring of the whiskers, it is possible to directly correlate the motion of an identified whisker with the firing of the cortical neurons that receive input from that whisker.

Connections of primary somatosensory cortex

Prominent reciprocal projections are found between primary somatosensory cortex and secondary somatosensory cortex, motor cortex, perirhinal cortex and thalamus. Barrel cortex also projects to striatum, thalamic reticular nucleus, zona incerta, anterior pretectal nucleus, superior colliculus, pons, red nucleus and spinal trigeminal brain stem nuclei (reviewed in (Aronoff, Matyas et al. 2010)).

The study of sensory processing in rats beyond the primary cortical fields is in its infancy. SI and SII send and receive dense reciprocal connections (Carvell and Simons 1987; Kim and Ebner 1999). It is an open question as to whether SI and SII in rodents function in a hierarchical manner as is believed to be the case in primates (Pons, Garraghty et al. 1992) or operate in parallel on different sorts of somatosensory information. The functional properties of the secondary field, SII, have been examined rarely, and only in anesthetized animals (Carvell and Simons 1986); other projects in the lab address this question.

1.3 Active sensing

Active sensing entails control of the sensor apparatus, in whatever manner best suits the task, so as to maximize information gain (Prescott, Diamond et al. 2011). It is purposive and information-seeking. Although the concept of sensor apparatus control applies to all modalities, it is perhaps most evident in the modality of touch.

The rat whisker-mediated sensory system is a prominent case of active sensing inasmuch as the rat precisely controls its whiskers. We argue that active sensing arises through two general modes of operation:

(1) *generative* mode, and
(2) *receptive* mode.

Generative mode

In the generative mode, the rat moves its whiskers forward and backward to actively seek contact with objects and to palpate the object after initial contact. The animal *causes* the percept by its own motion. Self-generated whisker motion is critical for wall following

(Jenks, Vaziri et al. 2010), distance estimation (Harris, Petersen et al. 1999), and identifying properties such as shape and size (Brecht, Preilowski et al. 1997; Harvey, Bermejo et al. 2001). As a rat or mouse feels its way through the world, it senses its own whisking (Ganguly and Kleinfeld 2004). From the relationship between the whisking cycle and the contact signal (Curtis and Kleinfeld 2009) the animal localizes objects with millimeter-precision (Knutsen, Pietr et al. 2006). The discrimination of texture (see Figure 1.4) is one condition in which rats generate neuronal sensory representations through their own whisker motion (Maravall, Petersen et al. 2007; von Heimendahl, Itskov et al. 2007; Diamond, von Heimendahl et al. 2008; Diamond, von Heimendahl et al. 2008; Khoshnoodi, Motiei-Langroudi et al. 2008; Lak, Arabzadeh et al. 2008; Mitchinson, Arabzadeh et al. 2008; Arabzadeh, von Heimendahl et al. 2009; Montani, Ince et al. 2009; Diamond 2010; Prescott, Diamond et al. 2011; Diamond and Arabzadeh 2012).

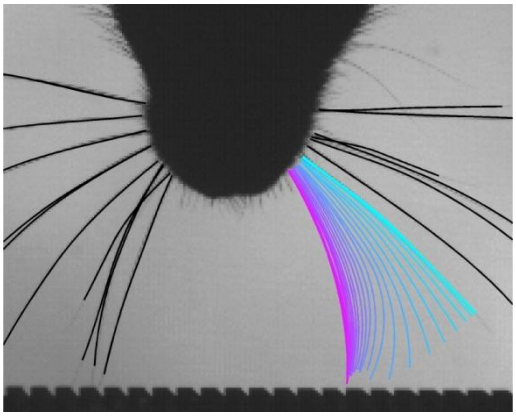


Figure 1.4. Active sensing – the generative mode. A rat identifies a textured plate by generating vibrations as the whiskers move along the surface. Whisker C4 is traced in color over sequential 1 ms steps. The image shows frame-to-frame tracking of whisker position as it gets stuck in a groove and is subsequently released at high velocity. From (Diamond and Arabzadeh 2012).

Receptive mode

It is difficult to quantify rodents' use of their whiskers in natural, out-of-laboratory settings. But even in the absence of objective data it seems reasonable to assume that some forms of perception rely on blocking motor output to keep the whiskers immobile. For example, how do rats perceive the passage of a large predator above their burrow? We speculate that they place their whiskers in contact with the walls and floor, with negligible whisking output, to "listen" for vibrations (see Figure 1.5).

We can further develop the illustration of the rat feeling for ground vibrations in the receptive mode. If the burrow's walls tremble, is the predator approaching (increasing vibration intensity) or moving away (decreasing vibration intensity)? Changes and differences in vibration intensity seem ecologically relevant, and it is exactly this form of perception that we have tried to bring from nature to the laboratory in this project.

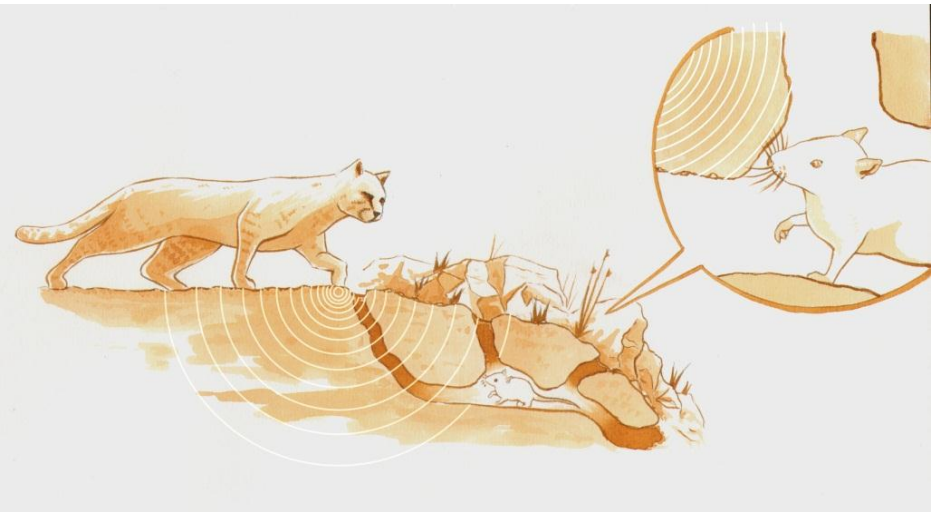


Figure 1.5. Active sensing – the receptive mode. As a predator approaches the rat's hiding place, the vibration signal might be transferred to the whiskers through their contact with the walls and floor of the burrow. Changes in vibration intensity over short time intervals would provide important information about the speed and direction of the predator. Drawing by Marco Gigante, SISSA Tactile Perception and Learning Lab.

It is tempting to name the state of the sensory system characterized by exploratory whisking as "active" and the state of quiet immobility as "passive" (Kleinfeld, Ahissar et al. 2006), but this nomenclature is misleading in its implication that the nervous system itself becomes passive in the immobile state, waiting to be subjected to unknown events. Observations collected in the present experiments suggest that the animal is highly "active" even when it places and holds its whiskers in contact with a moving stimulus. For this reason we refer to the "quiet" whisker state as the "receptive mode" rather than the passive mode.

To summarize, in the receptive mode, rats immobilize their whiskers to optimize the collection of signals from an object that is moving by its own power. The receptive mode – specifically, the perception of vibrations applied to the whiskers by external devices – will be the focus of the thesis.

Active sensing in humans

A discussion of human tactile perception is beyond the scope of this introduction, but it is interesting to note features that distinguish hand-mediated from whisker-mediated tactile perception, as well as features in common. Humans (and other primates) grasp and manipulate objects with their hands whereas rodents do not grasp or manipulate objects with their whiskers. (However, tactile information collected through the whiskers may be a precursor to grasping with the paw or mouth.) Moreover, human haptic perception relies to a great extent on proprioceptive signals from the joints and tendons. Proprioceptive signals of this sort are not present in the whisker follicles.

Common to human and rat tactile perception, we argue, is variation in the mode of operation according to the ongoing task. Humans adopt a broad range of sensorimotor strategies to collect information through the hands. These many regimes of acquisition are collectively referred to as "haptic exploration" (Lederman and Klatzky 1987). They range from following edges, palpating surfaces to detect texture and softness, and resting the fingertips on an object to detect vibration or motion (Jones and Lederman 2006).

If we need to check whether our computer has been turned off, we would likely place our fingertips lightly on the case to feel for vibrations produced by the fan. It is unlikely we would palpate the surface and sweep our fingertips along it, as we would do for a texture judgment (Gamzu and Ahissar 2001). Such motion can confound the skin vibration emanating from the computer fan with the skin vibration produced by motion along surface features. Thus, we (primates) adjust our hand and finger motor output according to what information we need to extract about the objects around us. In this thesis, the human psychophysical experiments are meant to capture the natural capacity of people to judge vibrations through receptive sensing.

1.4 Perception in rats?

The previous section freely uses the word "perception," yet the term itself is hard to define. A perceptual experience begins with the sensing of physical events, but it extends beyond the sensation. The percept is the sensation bundled together with the significance, or "meaning" of the sensation. Meaning depends on knowledge gained in previous experiences of that sensation; it depends upon the positive or negative expectations triggered by the sensation. Following the work of others, we take the view that perception is the process that transforms sensations into the experience of real objects. Perception makes sensations feel like they belong to things that are "out there" is the world, to use the term coined by Whitfield (Whitfield 1979). Inspired by literature that ends back 130 years (Munk 1881), we are persuaded that neocortex is the organ that endows simple sensations with the quality of belonging to objects.

The vibrissal sensory system of rodents has proven to be a spectacular platform for work on sensory coding, but is it suitable for the study of *perceptual* mechanisms? Until a few years ago, many neuroscientists would readily attribute perception to primates but would argue that rodents act in a more reflexive manner, by simply associating a specific stimulus with the response most likely to trigger a reward. This has changed as investigators have found that rodents can be trained to weigh sensory evidence (Kepecs, Uchida et al. 2008), to assess reward statistics, to express their level of confidence in the outcome of their choices (Lavan, McDonald et al. 2011), and even to generalize rules (Murphy, Mondragon et al. 2008), all in a primate-like manner. Rats spontaneously recognize views that differ by angle, size, and position as being instances of the same object (Zoccolan, Oertelt et al. 2009; Tafazoli, Di Filippo et al. 2012); such generalization is a hallmark of true visual perception, and was once believed to belong only to primates. All the work cited above indicates that the rodent brain processes physical signals in order to build up representations of objects and things that are

"out there" in the world, exactly the operation that Whitfield assigned to intracortical processing (Whitfield 1979).

A second function is implicit in the essay of Whitfield; the cortex is critical for the storage and recall of previous sensory experiences. The neuronal activity that encodes elemental sensory data can gain meaning only when it is integrated with memories of previous encounters with the same or different stimuli. Many behaviors require sensory information to be retained, whether in long term or short term (working) memory. Whereas neuronal activity in the ascending pathways to cortex and in primary sensory cortex itself subsides rapidly when a stimulus is removed, later stages of cortex seem to have a special capacity for retaining salient information (Romo, Hernandez et al. 2002). The work presented here introduces a new paradigm in which we show that rats can form parametric sensory working memories comparable to those of humans.

1.5 Sensory integration

One of the fundamental functions of cortex is to combine and integrate sensory information across time. This integration can be useful especially when the sensory inputs are noisy and unreliable. The relationship between enhancement of performance and processing time suggests whether, and how, subjects accumulate information over time in order to make accurate decisions.

The ability of monkeys and humans to integrate sensory information over time has been well established ((Mateeff, Dimitrov et al. 2000); (Roitman and Shadlen 2002); (Palmer, Huk et al. 2005)). However, in rats existing data is conflicting ((Stüttgen and Schwarz 2010); (Rinberg, Koulakov et al. 2006). Nevertheless whether this ability is used by subjects in any circumstance is not clear. In this study we introduce a new paradigm in which we compare the ability of human and rats to integrate sensory information for different periods of time and ask following questions: Can subjects achieve higher accuracy by integrating sensory information over time? Is this integration independent of subjects' coding/decoding strategies?

These questions are important for understanding the mechanisms by which the sensory system processes the information.

Behavioral reverse correlation

In psychophysics one attempts to correlate behavior to physical parameters of the sensory signal. This is a crucial step towards objectifying the subjective experience. The first attempt to answer this question dates back to the Fechner's book "Elemente der Psychophysik" (Fechner) in which he tried to correlate the physical and phenomenal worlds or, in other words, to establish causal links between physical features of the stimulus and perception. This relation gives us an opportunity to quantify how the brain processes and uses the sensory information. On the other hand we can speculate that different strategies would lead to different usage of this sensory input. How can we solve this puzzle?

Perception is an active process and in any perceptual tasks subjects try to exploit the most useful information coming from sensory signal. Sensory information thus should be extracted differently in different circumstances depending on the subjects' strategy. This strategy therefore would lead to different behavioral output. By carrying out a reverse correlation between the sensory signal and the behavioral output of the subject one can track this strategy. In other words, instead of determining the behavioral output for different physical parameters of the stimuli, instead one searches for the physical parameters that lead to an observed behavioral output.

1.6 Working Memory

Working memory is the short-term storage of information in the brain, and the use of that information immediately thereafter to solve a task; remembering a phone number for the time necessary to punch it into the keypad depends on *symbolic* or *semantic* working memory. Our interest here is in *sensory* working memory – the short term storage of quantifiable stimulus parameters. Sensory working memory has never been demonstrated in rats. Spatial alternation, odor or object-guided delayed match to sample procedures have been claimed to be tests of working memory in rodents, as they require active maintenance of information across a trial (Dudchenko 2004). However, to our knowledge, there is no systematic study demonstration of a parametric working memory task, with graded stimuli. Thus, in the existing literature, exactly what is being remembered cannot be defined in quantitative terms. As a consequence, the mechanism of information coding during memory maintenance remains unknown.

Forms of memory

To correctly perform a behavioral task, different types of memories are required. Reference memory is a memory for information, invariant across trials, upon which the "rules" of a given task must be applied. For example, a specific sound cue if followed by a specific action will always be followed by reward. Working memory, in contrast to reference memory, is typically a delay-dependent representation of stimuli that are used to guide behaviour within a task. Initial studies of working memory described it as a representation of a cue over a delay period in which the cue is not present, to make a subsequent response (Honig 1978). However, recent definitions, emphasized the "working" aspect of this type of memory; Eichenbaum and Cohen define working memory as a type of short-term memory that involves active manipulation by the individual (Eichenbaum and Cohen 2004)

Although David Olton and Werner Honig in the 1970s were the first researchers to apply the term working memory to the animal's short-term storage of information, earlier experimenters had devised "delayed reaction" paradigms to see how long a rat could remember a stimulus that was not present ((McAllister 1932), (Munn 1950)). For many years afterward, different "spatial" working memory paradigms were adapted that required rats to remember a location or set of locations, and either approach or avoid these locations subsequently (delayed alternation (Whishaw and Pasztor 2000); the radial arm maze

(Foreman and Ermakova 1998)). Later on, delayed match or non-matching to sample (DMS/DNMS) tasks were used (spontaneous exploration; (Ennaceur and Delacour 1988) DMNS with objects (Kesner, Bolland et al. 1993); DMNS with odors (Dudchenko, Wood et al. 2000).

Different challenges are associated with each of these paradigms. In all of the tasks listed above it is impossible to specify the precise content, and therefore the brain's coding, of the memory that is used to solve the task. There is no knowledge for instance of the definition of an object nor of the brain's representation of that object. DNMS tasks have the advantage that the experimenter specifies the to-be-remembered stimuli. However, postural mediation of the to-be-remembered response - which enhances performance but is not explicitly required by the task - can occur and is generally considered an obstacle to the measurement of memory (Panlilio, Yasar et al. 2011).

Electrophysiological evidence

In spite of several observations of the neuronal signature of working memory in the form of "delay activity" in monkey electrophysiology ((Fuster and Alexander 1971), (Sakai and Miyashita 1991), (Romo, Brody et al. 1999)), there is very limited data from rodent electrophysiology (Dudchenko, Wood et al. 2000). From available evidence it is hard to infer the mechanism underlying such phenomena at the neuronal or network level. Moreover, in none of the versions of working memory in rodents, a systematic study of sensory coding, the role and the degree of involvement of different sensory areas, has been done.

2. Behavioral Methods

2.1 Animal subjects

Seven male Wistar rats (Harlan) were housed individually or with one cage mate and maintained on a 14/10 light/dark cycle. They were water restricted and were trained to perform tactile discrimination tasks for a pear juice reward diluted with water (1 unit juice: 3 units water). The water restriction schedule allowed access to water *ad lubitum* for 1 h/d after each training session. The animals weighed about 300 g at the outset and gained weight steadily for several months.

2.2 Apparatus

The behavioral apparatus consisted of a custom-built plexiglass chamber measuring 25 × 25 ×38 cm (H × W × L) attached to a stimulus delivery port (Figure 2.1). In the front wall, a 4 cm diameter hole (labeled as head hole) allowed the animal to extend its head from the main chamber into the stimulus delivery port. Within the stimulus delivery port a small nose poke was centered in front of the rat. An infrared light emitting diode (LED) illuminated the stimulus delivery port to permit video recording. In some sessions, high speed video images (Optronis CamRecord 450) were taken at 1,000 frames per second through a macro lens (Kawa CCTV Lens, LMZ45T3) to monitor head and whisker position and movement during behavior.The rat received rewards of diluted fruit juice. A custom-made avr32 board (National Instruments, Austin, TX) was used to control the juice pump.

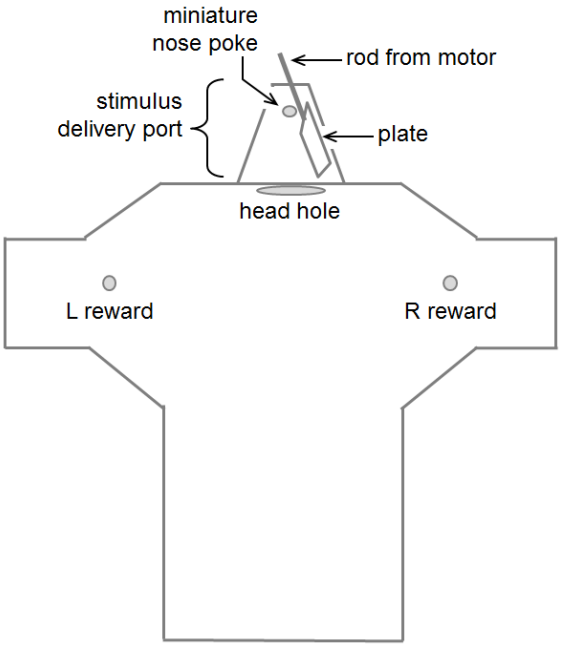


Figure 2.1. Schematic configuration of the apparatus viewed from above.

The stimulus delivery motor was a Bruel & Kjaer 4808 shaker which was placed on its flank in order to produce motion in the horizontal dimension. The motor was selected due to its ability to deliver translation of up to ±2 cm from the set point (4 cm range) with frequency content of 5Hz to 10 kHz, depending on the software filter. Since the motor was designed to allow constant acceleration across frequencies, its output was reduced in frequency for large displacements. Our stimuli included large displacements so it was convenient to use a filter, described below, to make the command to the motor compatible with its specifications.

A lightweight aluminum rod was fixed to the diaphragm of the shaker, projecting the motor's translation into the stimulus delivery port. On the end of the rod, a 20 x 30 mm plate with an approximately vertical orientation was attached. The rat received the stimulus by placing its whiskers on the plate with an approximately orthogonal orientation. Double-sided sticky tape was placed on the plate prior to each session to make the whiskers remain in contact and to "follow" the motor during stimulation. Using the miniature nose poke as a reward port during shaping, the rats learned to place their head between the head hole and nose poke; at this point, head movement was reduced and the natural position of the whiskers was to rest in contact with the stimulator plate.

2.3 Experiment control and stimuli

The experiment ran automatically using software written in Lab View (National Instruments). During the shaping sessions of the training procedure, experimenters set variables such as reward size, task difficulty (the difference between the two stimuli to be discriminated), and interstimulus delay according to the progress of the rat. Once the animal learned the task, the experiment could run without any manipulation by the experimenter. Nevertheless, the experimenter monitored the session to detect and react to tendencies such as left/right bias or satiety.

Stimuli were composed of a random series of velocity values ("noise") taken from a Gaussian distribution with 0 mean and standard deviation referred to as σ (Figure 2.2). The difference in velocity variance causes proportionate changes in position and acceleration variance and other parameters (derivatives or integrals). Each stimulus thus carried multiple features that could be extracted, in theory, to solve the task. The Results section addresses the question of which features actually contributed to the percept. As the features that humans and rats may use to solve the task are not known *a priori*, we denote each stimulus by its root parameter, velocity standard deviation, σ. In our experiment, σ ranged from 23 to 420 mm/s.

Whisker stimuli were delivered as rapid rostral/caudal deflections of the plate position in the stimulus delivery port. To obtain the velocity time series', a string of voltage values was transmitted to the motor after executing a Butterworth filter with 110 Hz dropoff. This filter assured that the input commands to the motor matched the manufacturer's specifications for the motor.

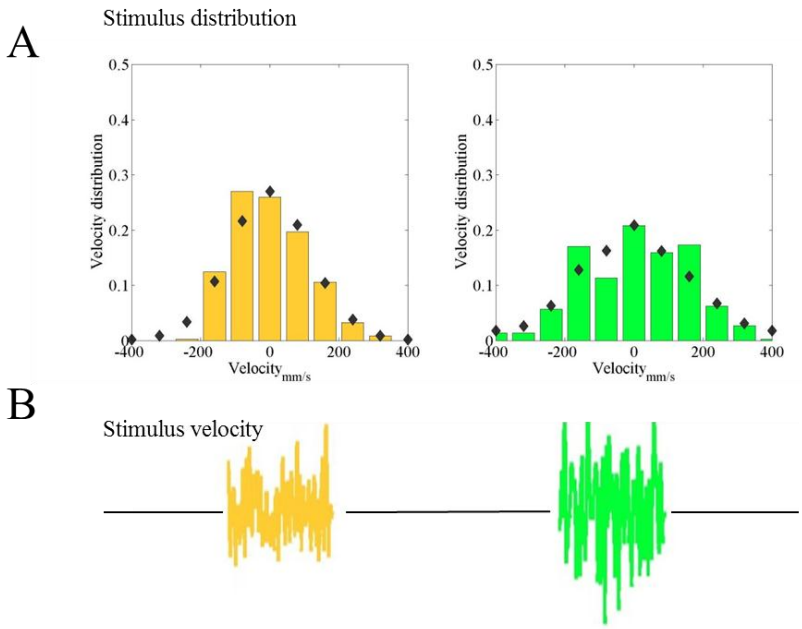


Figure 2.2. Tactile noise. (A) Stimuli were composed of a random series of velocity values ("noise") where the sampling probability of a given velocity value was given by a Gaussian distribution with 0 mean and standard deviation σ. For two discrete vibrations, the underlying probability density functions are shown in filled dark diamonds while the actual distributions of velocity are shown for single instances of σ. (B) The velocity time series are plotted for the instances of σ shown in (A).

Stimulus properties were verified by two techniques. First, a position transducer was fixed to the rod to measure all stimuli on-line. Second, high-speed video clips (1,000 frames/second) were recorded during playback of the entire stimulus library. A custom-made program tracked and analyzed plate motion. All descriptions of the stimulus are based on the verified output of the motor.

2.4 Behavioral task in rats

The behavioral task is illustrated in Figure 2.3. The final goal of training was to have the rat place its snout in the stimulus delivery port to initiate the trial, receive vibratory whisker stimuli (Figure 2.3A), and then withdraw (Figure 2.3B) to select one of two reward ports (Figure 2.3C).

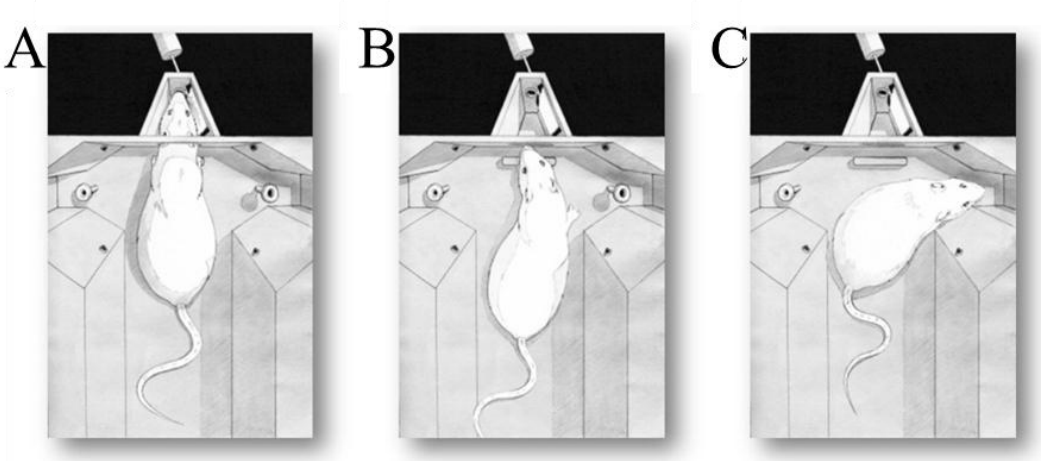


Figure 2.3. Setup and the behavior. (A) At the start of each trial, the rat extends its head into the stimulus delivering port. The trial starts when the snout is stable in the nose poke. **(B)** At the conclusion of the two stimuli a "go" cue is sounded and the rat withdraws and (C) collects juice at the reward spout.

To accomplish this behavior we developed an extensive shaping procedure, as described below. Over a period of 8 to 12 weeks, we led each rat through six phases.

Shaping Phase 1. 2-3 days before being placed in the apparatus, the rat was put on a water-restriction schedule with 1h/d water access *ad libitum*. Then it was habituated to the environment of the apparatus. In this phase, the rat also learned that juice could be obtained from the Left and Right reward delivery spouts (Figure 2.1).

Shaping Phase 2. The rat was trained to extend its head from the main compartment into stimulus delivery port. The distance between head hole and nose poke (Figure 2.1) was around 4 cm and could be adjusted to each rat. Head movement was monitored using high-speed video upon which a custom algorithm operated on-line to detect head movement. Morevover, the nose poke contained an optic sensor illuminated by an infrared photo beam, broken by the tip of the snout. We induced the rat to place its snout in the nose poke by offering it a hand-held juice dropper just external to the nose poke. Then on each instance when (i) it triggered the nose poke optic sensor, and (ii) head movement was below a user-set threshold for at least 50 ms, a "go" cue was sounded and the rat was led from the stimulus delivery port to the reward spout where it collected a drop of juice.

Each reward delivery spout was equipped with an infrared LED-based sensor to detect the presence of the animal. In order to receive a juice reward rat had to break the light beam positioned in front of the reward delivery spout. A 50 µl drop of juice was delivered via syringe pump (NE-500 programmable OEM, New Era Pump Systems, Inc. NY USA). They learned this step (nose poke / "go" cue / reward collection) in a single session.

Shaping Phase 3. In this set of sessions, the time required in the nose poke before presentation of the "go" signal was gradually increased. 4-5 sessions were required until the desired waiting time, 8,000 ms, was reached.

Shaping Phase 4. For the first time, the motor was enabled and the rat began to perform a whisker-mediated task. The continuous, quiet presence of the rat in the nose poke for 300 ms triggered the delivery of a pair of stimuli of 500 ms duration each, separated by 200 ms. The rat was required to receive two stimuli, as defined in Table 2.1.

Table 2.1. Nomenclature for the two stimuli presented in each trial.

Name	Temporal order	Velocity standard deviation
Base stimulus	first	σ1
Comparison stimulus	second	σ2

The "go" cue was sounded only at the end of the second stimulus; if the rat withdrew before the sound cue, no reward was released at the spout ("aborted trial"). The rat had to select the Right or Left reward spout depending on relative standard deviations of the two stimuli. For instance, the rule for one rat, fixed across all sessions, might be if σ1>σ2 go right, if σ1<σ2 go left.

Clearly the discrimination becomes more difficult as the difference between σ2 and σ1 becomes smaller. In Phase 4, the task began with large differences. To characterize the distance between the two stimuli we define the Standard Deviation Index (SDI):

Equation 2.1:

$$SDI = \frac{\sigma 2 - \sigma 1}{\sigma 2 + \sigma 1}$$

SDI is 0 when the base and comparison stimuli have equal expected values of velocity standard deviation, although since the stimuli were stochastic no two stimuli were in fact equal. SDI = 0.33 and -0.33 corresponds to the case where the comparison σ is, respectively, twice as large or twice as small as the base σ.

Note that SDI is scaleless and, if performance were to vary according to the size of the SDI across the full scale of σ, from "weak" to "strong" base/comparison pairs, this would suggest that the sensory system detects differences according to the Weber Law.

Shaping phase 5. The purpose of this phase was to improve the subject's accuracy. At the beginning of this phase, we used large SDIs (e.g. SDI = +/-0.35) which gives a Weber fraction of approximately 110%. After animals reached a mean performance of 80% correct over all pairs, the SDI was reduced progressively.

Shaping phase 6. If the base stimulus were fixed and only the comparison stimulus varied (with σ above or below that of the base on each trial), the rat might learn to ignore the base stimulus and solve the task only by applying a threshold to the comparison stimulus. To ensure that the rat compared the two stimuli, the stimulus set was constructed so that each base stimulus could be followed by a comparison with either higher or lower σ (Figure 2.4). In this way subjects were forced to learn to compare the two stimuli. To solve the task, the rat must encode the base stimulus, store it as a memory trace, and then encode the comparison stimulus and compare it to the memory trace of the base stimulus. We refer to this experimental design as ***Comparison Generalization Pair Design*** (CGPD). In a form comprising 10 stimulus pairs, the set of stimuli would be represented as in Figure 2.4A, where each square is a unique (σ1, σ2) combination. The dashed diagonal line represents σ1 = σ2, so that all stimulus pairs on one side of the diagonal are associated with the same action. This design was used for testing whether the rat truly learned the comparison task. Henceforward the training method split into two protocols, for two different sets of rats:

1. In four rats, we set out to quantify the possible relationship between physical parameters of stimulus and behaviour through psychophysical methods. Accordingly we kept the base stimulus fixed while varying the target stimulus (Figure 2.4B). To ensure that rats did not shift to a strategy of merely applying a threshold to the comparison stimulus, 20% of trials exploited the full stimulus set according to the CGPD routine. The rats performed well on all stimuli, indicating that they continued to use the correct comparison rule even though 80% of trials had the same base stimulus σ.

2. In three rats, to test the working memory capacity, CGPD was used with a large standard deviation index (SDI = 0.35). The delay between stimuli was gradually increased from 200 ms to 6,000-8,000ms. It should be noted that the σ range and number of pairs used to study working memory was slightly more limited in rats than in humans.

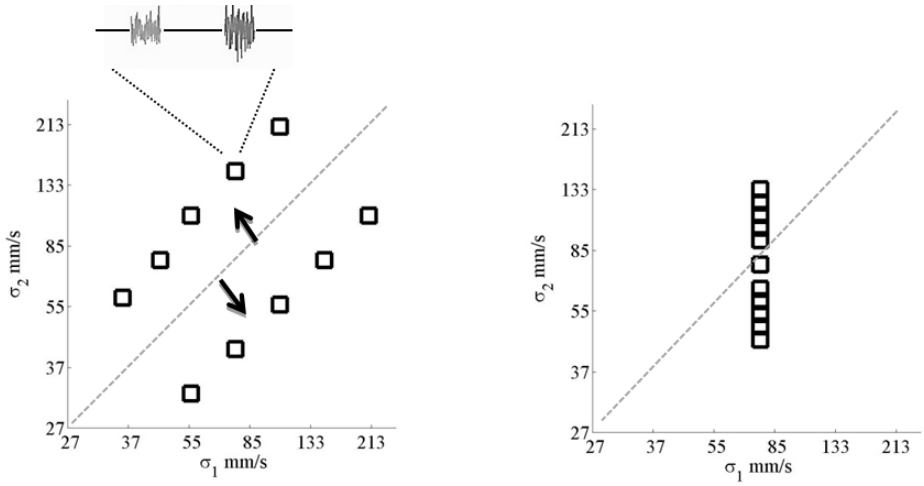


Figure 2.4. Stimulus sets. (A) Comparison Generalization Pair Design (CGPD) used to explore working memory. (B) Fixed base stimulus designed used to map the psychometric function.

The following are the key advantages of the CGPD:

i. Any given value of σ1 can be *followed*, with equal likelihood, by two different values of σ2.
ii. Any given value of σ2 can be *preceded*, with equal likelihood, by two different values of σ1.
iii. The complete range of σ can be made very large.
iv. The large set of σ values in one session make each trial "unique" and must be encoded on-line rather than recalled from reference memory.

These advantages, taken together, constitute an experimental structure that guarantees working memory. If the rat were to operate according to any rule that did not involve a direct comparison between σ1 and σ2, accuracy would be very low. For example, if the rat were to attend to the second stimulus and decide to turn right if σ2 > 85 mm/s, performance would be close to chance. Thus, we argue that good performance in the CGPD condition indicates that the rat is using working memory and is following a general rule applied across the entire stimulus dimension. The cognitive structure of the task is summarized in Figure 2.5.

Phase 6 usually required about 4-8 weeks of training.

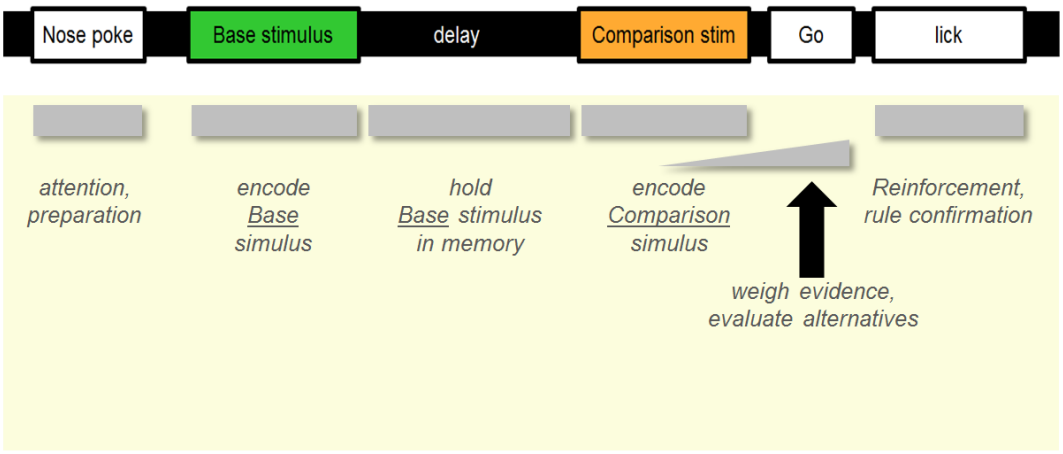


Figure 2.5.Cognitive structure of a single trial.

2.5 Controls

The motor vibration generated acoustic signals that were easily heard by the human auditory system, and could provide clues as to the comparative values of σ in a trial. In theory these sounds would not be expected to be accessible to rats because their frequency was below 200 Hz, well below the sensory range of rats (Kelly and Masterton 1977). Nevertheless, it was important to verify that meaningful signals were acquired *only* through the whiskers. To rule out stimulus information related to the acoustic noise of the motor, two control conditions were used: (1) the motor was detached from the plate in the stimulus delivery port, (2) the adhesive surface was removed from plate so that whiskers slipped along the plate and no

longer "followed" the motor. In both conditions auditory cues remained without whisker motion, and the performance of all rats dropped to the chance level.

We did *not* clip off the whiskers as a test, because this would lead to general disorientation and would not be a specific test of whisker use in the task.

2.6 Human subjects

21 human subjects (9 males and 12 females; aged 22-35) were tested. They signed informed consent and were introduced to the purpose of the experiment (Appendix 1).

2.7 Apparatus

Subjects sat in front of a computer monitor and rested their left arm and hand on a firm cushion. The left index finger was placed in contact with the tip of a probe driven by the same motor used in rat experiments (Bruel & Kjaer 4808 shaker). They wore headphones that presented acoustic noise and eliminated ambient sounds. The setup is depicted in Figure 2.6.

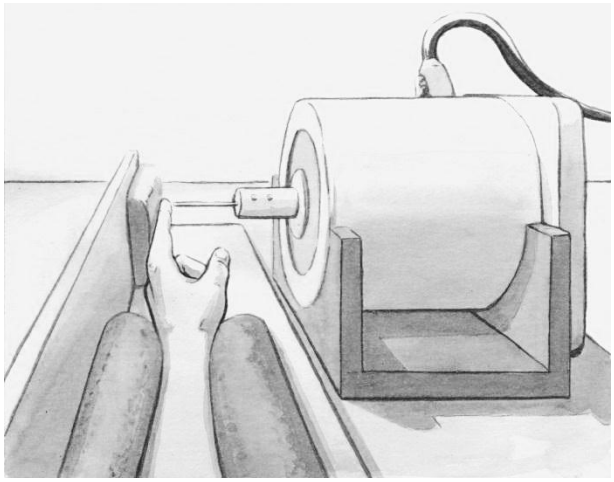


Figure 2.6. Human psychophysics setup. Participants performed the same discrimination task as rats, by holding their left index fingertip in contact with the tip of a vibrating rod and responding by pressing left or right arrow keys on a standard keyboard. They received correct/incorrect feedback from the monitor on each trial.

2.8 Experiment control and stimuli

The same stimuli used in rats were delivered to the subject's fingertip except that the velocity/position dimension was reduced by a factor of 2. This is because stimulus energy is delivered directly to the skin, unlike the case in rats where the interposed whisker shaft absorbs much energy.

2.9 Behavioral task in humans

See Appendix 1 for instructions to the subject.

Each trial started when the subject used her right hand to press the 'up' arrow key on a standard keyboard placed in front. After the base and comparison stimuli were delivered to the left index fingertip, a "go" cue appeared on monitor and the subject responded by pressing 'left' or 'right' arrow keys. The rule, not stated explicitly to the subject, was that the 'left' button was correct if $\sigma1<\sigma2$ while the 'right' was correct if $\sigma1>\sigma2$. Feedback was provided with different colors flashed onto the monitor after the response (red: incorrect and green: correct). The training session consisted of a rule finding period in which the CGPD stimulus pairs were used. This phase continued until the subject felt that she had deduced the rule and performed a minimum of 100 trials with >85% correct. Each subject was then asked to write down their best interpretation of the rule (e.g. "press the 'left' arrow if the first stimulus is stronger, "press the 'right' arrow if the second stimulus is stronger."). After the training session, data collection began.

2.10 Data analysis

Psychometric curves

The data from rats and humans were processed by the same algorithms. We computed psychometric curves using only trials with same stimulus durations. For each data point we computed the proportion of trials in which subjects reported $\sigma2>\sigma1$. Ideal response values would be 0% for negative SDIs (i.e. where $\sigma2<\sigma1$) and 100% for positive SDIs (i.e. where $\sigma2>\sigma1$). In other words, the perfect psychometric function would resemble a step function with a discontinuity at $\sigma2=\sigma1$.

Of course, real organisms are never ideal, so psychometric curves resemble sigmoid functions. Therefore we fit the response plots with a 4 parameter logistic function using maximum likelihood method in Matlab (MathWorks) where *y* is percent called $\sigma2>\sigma1$ as a function of *x*, the SDI.

Equation 2.2:

$$y = \frac{Min - Max}{1 + \left(\frac{x}{x_0}\right)^{dx}} + Max$$

The four parameters are: *Min* – the lower asymptotic, *Max* – the upper asymptotic, x_0 – the inflection point along the SDI axis, and *dx* – the slope factor of the curve. The actual slope is calculated by using $x=x_0$ on the derivative of *y* and it follows that

Equation 2.3:

$$Slope = dx\frac{(Max - Min)}{4x_0}$$

To perform statistical tests of the effect of stimulus duration on different parameters of psychometric fit, we shuffled the comparison stimulus duration tags and computed the best linear fit on 3 different stimulus durations derived from shuffled distribution. We computed the best linear fit on parameters derived from empirical data and compared the slope of the linear fit from empirical data to the shuffled distribution. The statistics on parameters of fit was calculated using resampling methods (Wichmann and Hill 2001).

Psychophysical reverse correlation

To perform a comparison between a subject's (rat or human) trial-by-trial choices and the choices of an ideal observer who perfectly extracts information about a selected feature, we applied a simple measure, the "normalized Hamming distance". It first measures the number of substitutions required to change the subject's choices across a sequence of trials into the sequence of choices that would be made by an ideal observer who perfectly extracted the selected feature. Then the number of substitutions is normalized by the total number of trials for that condition.

Equation 2.4:

$$normalized\ Hamming\ distance = \frac{1}{n}\Sigma|a_i\_b_i|$$

$$a_i = \begin{cases} 1 & if\ \ feature(\sigma 2) - feature(\sigma 1) > 0 \\ 0 & if\ \ feature(\sigma 2) - feature(\sigma 1) < 0 \end{cases}$$

$$b_i = \begin{cases} 1 & if\ \ animal\ responded\ \sigma 2 > \sigma 1 \\ 0 & if\ \ animal\ responded\ \sigma 2 < \sigma 1 \end{cases}$$

Where a_i and b_i are the *i*th argument of the "subject choice" sequence (i.e. *i*th trial) and ideal observer outcome sequence, respectively.

If no substitutions are required, the normalized Hamming distance is 0 for that feature, in which case the subject is presumed to have used precisely that feature to make its choices. If the normalized Hamming distance is 0.5, then the feature of interest would appear to have no influence on the subject's choices. In this analysis only trials with SDIs of 0, -0.05 and 0.05 were used.

Forty one features were extracted from each stimulus and tested (Table 2.2)

Feature number	**Physical quantity**	**Measured as**
1	Position standard deviation	$\sigma(position)$
2	Velocity standard deviation	$\sigma(V)$
3	Acceleration standard deviation	$\sigma(a)$
4	Speed standard deviation	$\sigma(\|V\|)$
5	Summated values of Speed	$\Sigma(\|V\|)$
6	Number of positive velocity outlier events	$N^o\ V > \bar{V} + 2\sigma(V)$
7	Number of positive velocity outlier events per duration	$\frac{N^o\ V > \bar{V} + 2\sigma(V)}{t}$
8	Summated values of positive velocity outlier events	$\Sigma\ (V > \bar{V} + 2\sigma(V))$
9	Summated values of positive velocity outlier events per duration	$\frac{\Sigma\ (V > \bar{V} + 2\sigma(V))}{t}$
10	Number of negative velocity outlier events	$N^o\ V < \bar{V} - 2\sigma(V)$
11	Number of negative velocity outlier events per duration	$\frac{N^o\ V > \bar{V} - 2\sigma(V)}{t}$
12	Summated values of negative velocity outlier events	$\Sigma\ (V > \bar{V} - 2\sigma(V))$
13	Summated values of negative velocity outlier events per duration	$\frac{\Sigma\ (V > \bar{V} - 2\sigma(V))}{t}$
14	Number of positive acceleration outlier events	$N^o\ a > \bar{a} + 2\sigma(a)$
15	Number of positive acceleration outlier events per duration	$\frac{N^o\ a > \bar{a} + 2\sigma(a)}{t}$
16	Summated values of positive acceleration outlier events	$\Sigma\ (a > \bar{a} + 2\sigma(a))$
17	Summated values of positive acceleration outlier events per duration	$\frac{\Sigma\ (a > \bar{a} + 2\sigma(a))}{t}$
18	Number of negative acceleration outlier events	$N^o\ a < \bar{a} - 2\sigma(a)$
19	Number of negative acceleration outlier events per duration	$\frac{N^o\ a > \bar{a} - 2\sigma(a)}{t}$
20	Summated values of negative acceleration outlier events	$\Sigma\ (x > \bar{a} - 2\sigma(a))$
21	Summated values of negative acceleration outlier events per duration	$\frac{\Sigma\ (x > \bar{a} - 2\sigma(a))}{t}$

22	Number of of positive position outlier events	$N^o\ x > \bar{x} + 2\sigma(\sigma)$
23	Number of of positive position outlier events per duration	$\frac{N^o\ x > \bar{x} + 2\sigma(\sigma)}{t}$
24	Summated values of positive position outlier events	$\Sigma\ (x > \bar{x} + 2\sigma(x))$
25	Summated values of positive position outlier events per duration	$\frac{\Sigma\ (x > \bar{x} + 2\sigma(x))}{t}$
26	Number of negative position outlier events	$N^o\ x < \bar{x} - 2\sigma(x)$
27	Number of negative position outlier events per duration	$\frac{N^o\ x > \bar{x} - 2\sigma(x)}{t}$
28	Summated values of negative position outlier events	$\Sigma\ (x > \bar{x} - 2\sigma(x))$
29	Summated values of negative position outlier events per duration	$\frac{\Sigma\ (x > \bar{x} - 2\sigma(x))}{t}$
30	Summated values of position	$\Sigma(x)$
31	Summated values of velocity	$\Sigma(V)$
32	Summated values of acceleration	$\Sigma(a)$
33	Summated values of position per duration	$\frac{\Sigma\ (x)}{t}$
34	Summated values of velocity per duration	$\frac{\Sigma\ (V)}{t}$
35	Summated values of acceleration per duration	$\frac{\Sigma\ (a)}{t}$
36	Summated values of absolute position per duration	$\frac{\Sigma\ (\lvert x \rvert)}{t}$
37	Summated values of speed per duration	$\frac{\Sigma\ (v)}{t}$
38	Summated values of absolute acceleration per duration	$\frac{\Sigma\ (\lvert a \rvert)}{t}$
39	Mean position	$\bar{x}$
40	Mean velocity	$\bar{v}$
41	Mean acceleration	$\bar{a}$

Table 2.2. Stimulus features used to calculate Hamming distance and choice probability. Features 39-41 are a control as their value in each vibration is around 0.

2.11 Appendix 1. Instructions to subjects.

In this experiment, you will receive on each trial a pair of vibrations on the index finger of your left hand. Your task is simply to compare the vibrations and indicate your choice by pressing a key with your right hand. You should press the left-arrow key to indicate the first stimulus of the pair and the right-arrow key to indicate the second stimulus. If the choice panel illuminates green, you made the right choice; red means the wrong choice. When the computer is ready to deliver the next trial, the blue panel will illuminate. Press the up-arrow when you are ready for the stimuli.

The entire experiment, start to finish, will last about 90 minutes. Between blocks of trials, you can pause for a break. Of course, if at any time you feel tired or uncomfortable, you can stop the experiment and let us know.

The warm up session has two purposes – first, to let you get comfortable with the stimulus. Find a position for your arm and hand that feels good and that you can hold for a while. Also, find the degree of pressure to apply to the probe that you think best lets you feel the features of the stimulus.

The second purpose of the warm up session is to allow you to discover the comparison rule. We will not tell you the rule for the comparison. When you feel a difference between the two stimuli, select one stimulus and the computer will tell you if you were correct. By trial and error you will be able to sense which properties of a stimulus you should feel in order to make a choice. When we see that you have correctly answered a string of trials, we will stop the warm up and ask you to verbalize the rule for stimulus selection. A verbalization will be something like: "Of the two successive stimuli, I need to indicate which one is _____."

After the warm up session, you will continue with further blocks of trials. Remember that between blocks of trials, you can pause for a break and if, at any time you feel tired or uncomfortable, you can stop the experiment and let us know. Please try to pay attention to each and every trial!

The standard payment for your time is 15€. To help motivate you to attend to every trial, you will receive a 5€-10€payment bonus if your overall performance across the entire experiment is greater than 75%-80%.

3. Results of the behavioral study

3.1 Overall performance

This chapter presents a behavioral study of tactile perception. We tested rats and humans on a delayed comparison task. Subjects were required to detect the difference between two successive vibrations delivered to the whiskers, in rats, or to the fingertip in humans, with a pause inserted between the two stimuli. After a training period of approximately 6 weeks, rats carried out 100–300 trials per day with stable performance. Human subjects (with only a cursory training period of 15 minutes) carried out 600-1200 trials in two to four sessions. Both human and rat subjects performed well. Four out of 5 rats learned the task, where the criterion for successful learning was at least 70% correct across ~5 successive sessions on the version of the task that used

- ***Comparison Generalization Pair Design*** (CGPD, the full scale of stimulus pairs (see Methods)),
- interstimulus delay of 800 ms.

Ten out of 11 human deduced the rule correctly during the initial training phase and performed above 80% correct in single sessions. As expected, performance improved as a function of the difference between the two comparison stimuli, so the performance in one session included a mixture of trials that were easy and difficult. On average, across all stimulus pairs, human subjects reached a mean performance of ~85% correct whereas rats performed on average at ~80% correct (Table 3.1).

3.2 Quantitative characterization of performance

The psychometric curve is a systematic way to assess performance, for it considers discrimination accuracy in relation to the difference between stimuli. To generate a psychometric curve, the data are selected from one subset of the full stimulus set, corresponding to trials in which $\sigma 1$ has a fixed value while $\sigma 2$ varies (Figure 2.3).

To illustrate the data, the abscissa gives the difference between the velocity standard deviations of the two vibrations, $\sigma 2 - \sigma 1$, normalized by their sum. Thus, abscissa values progressively farther to the right of 0 correspond to progressively larger differences in stimulus intensity, with the *comparison* stimulus greater; abscissa values progressively farther to the left of 0 correspond to progressively larger differences in stimulus intensity, with the *base* stimulus greater. The ordinate gives the percent of trials in which the subject judged the comparison stimulus as stronger ($\sigma 2 > \sigma 1$). Perfect performance would result in a step function from 0% to 100% as the abscissa values go from negative to positive. Since performance is never perfect, real psychometric functions are not step functions but are sloped curves known as *sigmoid* functions. We fit the psychometric curves with 4 parametric logistic functions (see Methods). The plots of Figure 3.1A illustrate the

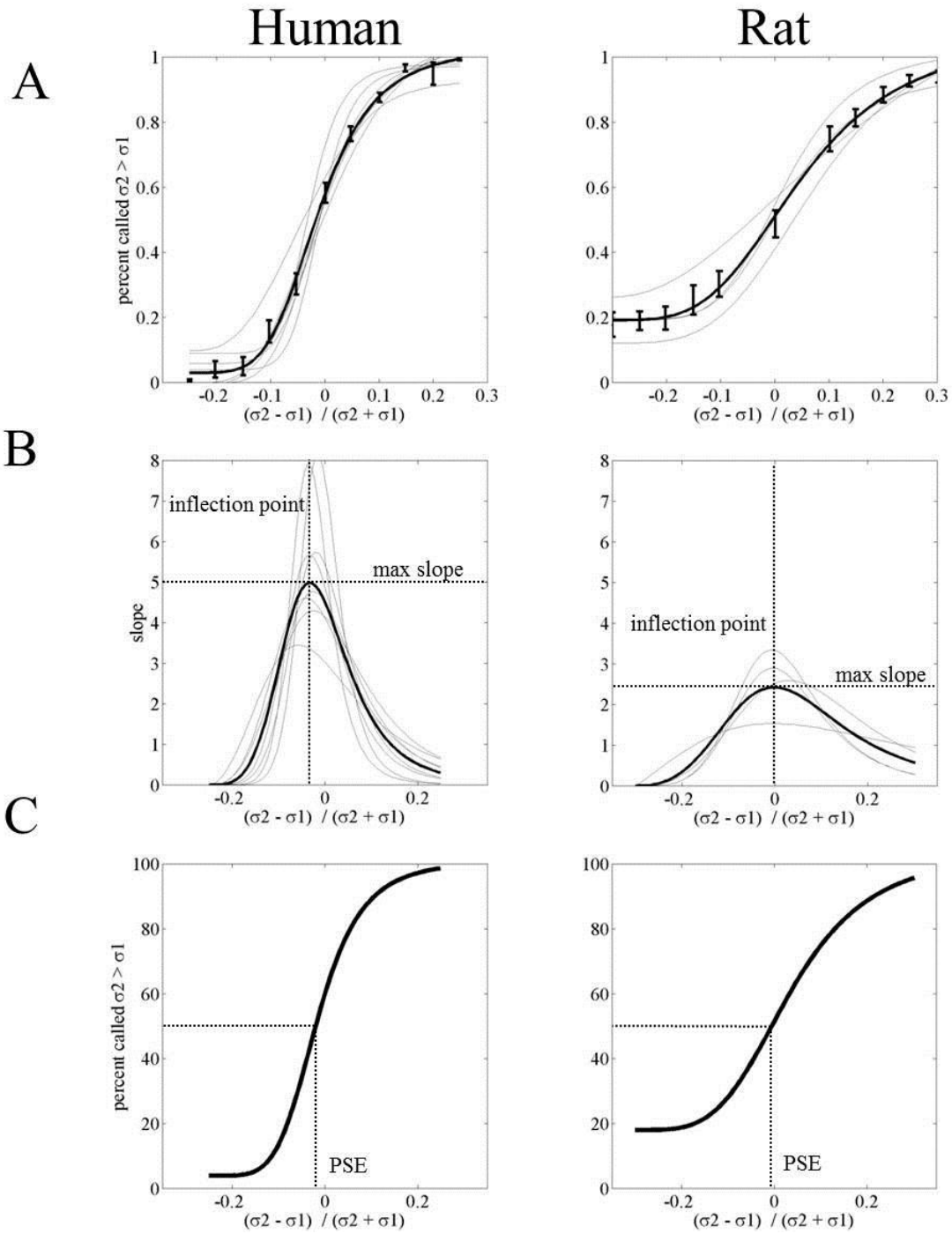


Figure 3.1. Subjects' performance. Psychometric curves are plotted for conditions when base and comparison stimuli were both 400 ms long. **(A)** Curves describe the performance of each subject (thin gray lines, 9 human and 4 rat subjects) and average over all subjects (thick black line). The average plots come from 8,815 trials for humans and 25,856 trials for rats. Error bars are standard error of the mean over subjects. **(B)** Derivatives of the psychometric curves. Horizontal dashed lines show the average peak slope while vertical dashed lines show the inflection point. **(C)** The sigmoid curves of panel (A) are re-plotted and the subjects' point of subjective equivalence is derived.

fitted psychometric functions of the humans (left) and rats (right) on trials when the duration of the base and comparison stimuli were 400 ms and the interstimulus delay was 800 ms. The

average performance is the thick black line and the single subject performance (9 humans and 4 rats) is the set of light grey lines. Overall, conspecific subjects had similar performance. The performance of humans was better than that of rats, as evidenced by the endpoints closer to 0 and 100, and by the steepness of the sigmoid function.

The steepness of the psychometric function is one key measure of sensory acuity. Figure 3.1B show the derivatives (see Materials and Methods) of the single-subject sigmoid functions (Equation 2.2)and the group averages, computed as:

slope = (percent change in probability of judging σ2 > σ1) / (change in (σ2- σ1) / (σ2- σ1)).

It is clear that humans tended to have higher peak values of slope than did rats. However, one of the four rats achieved a peak slope of about 3, which was equivalent to one human subject. The ranges of rat and human vibration acuity are thus overlapping. This panel also reports the inflection point, defined as the value of the abscissa, (σ2-σ1) / (σ2-σ1), where the psychometric curve slope changes from increasing to decreasing.

Panel C of the same figure illustrates the human (left) and rat (right) point of subjective equivalence (PSE), as derived from the average psychometric curve. PSE is defined as the relative values of σ1 and σ2 for which the subject perceives the two stimuli as being equivalent – that is, for these stimulus values the subject is equally likely to judge σ1 as greater and σ2 as greater. If the sigmoid plot were symmetric with respect to 180-degree rotation, the PSE and inflection point would be the same. Under the stimulus conditions illustrated here, the PSE was aligned closely to an abscissa value of 0, meaning that human and rat subjects tended to feel the base and comparison stimuli as equivalent when they were, in fact, equivalent. This implies that stimulus order did not affect the way they were perceived.

3.3 Accumulation of stimulus information over time

Many studies of tactile stimulus perception have utilized periodic, repetitive trains of stimuli to the fingertip (Luna, Hernández et al. 2005) or whiskers (Stüttgen and Schwarz 2010). For periodic stimuli with a fixed duration, all information is available in the first cycle, and additional cycles can improve sensation only through signal redundancy (Figure 3.2). The stochastic stimuli used in the present study – like those used in motion direction studies (Britten, Shadlen et al. 1992) (Britten, Shadlen et al. 1992) and in disparity discrimination tasks (Nienborg and Cumming 2007) – have a fundamentally different time structure. Since the vibration feature present at any given instant is sampled from a normal distribution, the essential properties of the stimulus – it's central tendency and variance – cannot be perfectly inferred from a short time window even by an ideal observer. Rather, these properties can be extracted with increasing precision as the stimulus continues over time. Because the information available to an ideal observer accumulates over time, subjects' performance in relation to vibration duration provides insights into whether and how the neuronal representation of the stimulus integrates information over time.

Figure 3.2. Periodic stimuli. Two examples of repetitive, regular vibrations, adapted from the publications of R. Romo. All information, except total stimulus duration, is present in the first deflection cycle, and successive cycles provide redundant information.

In short, how do rats and human subjects accumulate sensory information over time? To answer this question, we manipulated stimulus duration while monitoring the behavior. Theoretically, longer stimuli provide more information to the subject and should support enhanced performance. In contrast short stimuli provide less information and should give rise to reduced performance. This scenario holds only if the subjects make stimulus judgments by accumulating sensory information over time. In order to monitor the effect of duration on behavior, we computed psychometric curves separately for different stimulus durations. The design of the experiment is illustrated in Figure 3.3A. Interstimulus interval was always 800 ms. the base stimulus was always 400 ms in duration, but the comparison stimulus duration varied randomly across trials, with durations of 200 ms, 400 ms, or 600 ms.

If the subjects were to accumulate sensory information over time in this range of durations, then the slope of the psychometric function should increase as a function of stimulus duration. Figure 3.3B shows mean psychometric curves, fit over all subjects, for three different comparison stimulus durations (humans on left and rats on right). A clear difference between the results of humans and rats emerged. In human subjects, the curves related to different stimulus durations all had the same form, but were distributed laterally, giving the appearance of "parallel" plots. In rats, the curves related to different stimulus durations were all aligned in the horizontal dimension but had different forms.

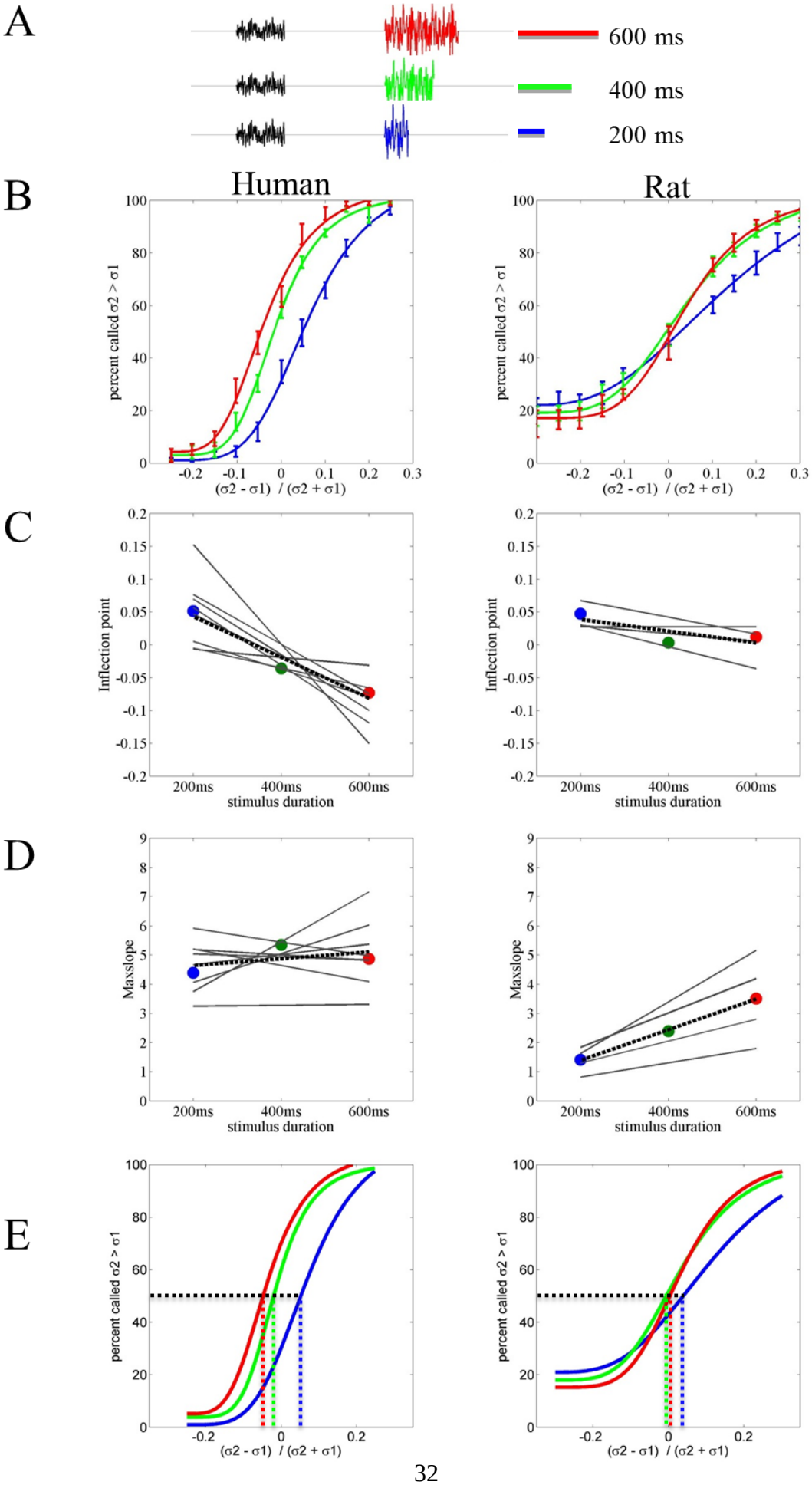
In order to quantify the effect of stimulus duration of the psychometric curve, we derived the same two diagnostic parameters as in Figure 3.1B, maximum slope and inflection point. Then we computed the best linear fit to those parameters as a function of stimulus duration (Figure 3.3C and D). If the slope of the best linear fit for a given parameter deviates significantly from zero, it indicates a significant effect of stimulus duration on that parameter (p value was calculated using a resampling algorithm; see Methods). Figure 3.3C shows the linear fit to psychometric curve slope as a function of stimulus duration. The slope of the psychometric curves of human subjects showed no significant change as stimulus duration increased (p=0.395 for average over all human subjects; see Table 3.1). However, the slope of the psychometric curves of rats showed a significant increase (p= 0.032 for average over all rats; see Table 3.1 for p values for individual rats). In short, human subjects did not improve their performance as comparison stimulus duration increased in the range of 200 to 600 ms, whereas rats gained knowledge from an increase in stimulus duration.

Next, in Figure 3.3D, we consider the linear fit to psychometric curve inflection point as a function of stimulus duration. The psychometric curves of human subjects exhibited a significant shift to the left as stimulus duration increased (p<0.001; see Table 3.1), indicating that lengthening the comparison stimulus caused σ to be perceived as larger. In other words, when $\sigma 1 = \sigma 2$ human subjects tended to report $\sigma 2 < \sigma 1$ if the comparison stimulus was *shorter* than the base stimulus, but tended to report $\sigma 2 > \sigma 1$ if the comparison stimulus was *longer* than the base stimulus. In rats, there was only a minor change in the sigmoid's inflection point when stimulus duration was changed. Overall, the change was not significant (see Table 3.1). Thus, rats' did not perceive σ as shifting when stimulus duration varied.

Finally, in Figure 3.3E we estimated the point of subjective equivalence (PSE), a parameter correlated with inflection point. In humans (left panel), because the PSE fell at about +0.065 for a 200 ms comparison stimulus and at about -0.035 for a 600 ms comparison stimulus relative to PSE for 400ms stimulus, we can surmise that for a subject to feel the base and comparison stimulus as having an equivalent value of σ, a 200 ms comparison stimulus would need to have a value of σ boosted by about 13% above that of the base stimulus. In contrast, to have equivalent perceived intensity, a 600 ms comparison stimulus would need to have a value of σ reduced by about 7% below that of the base stimulus. In rats (right panel), there were no signficant effects of stimulus duration on PSE.

In summary, humans appear to accumulate stimulus information up to some duration equal to or less than 200 ms; for longer durations, no additional information is acquired. Further experiments will be required to determine the time course of evidence accumulation for shorter stimuli. But the data in hand present an intriguing and counter-intuitive finding – under these conditions, humans cease to acquire knowledge about stimulus statistics within a short time frame (less than or equal to 200 ms); rats acquire stimulus statistics over longer durations – up to at least 600 ms. Most sensory system neuroscientists would have wagered the opposite – that rats collect information for short windows and humans for long.

The other principal finding is that humans overestimate σ for longer stimuli and underestimate σ for shorter stimuli. Rats do not shift the estimate of σ according to stimulus duration, but they do form a better representation of the value. This suggests that humans *summate* the relevant features over time, whereas rats *average* over time. The different sensory system strategies for accumulating evidence will be modeled and discussed in later sections.


A
600 ms
400 ms
200 ms
B
Human
Rat
percent called σ2 > σ1
(σ2 - σ1) / (σ2 + σ1)
C
Inflection point
200ms
400ms
600ms
stimulus duration
D
Maxslope
E

PRECEDING PAGE

Figure 3.3 Effect of stimulus duration of performance. (A) A sample trial with 600 ms (red), 400 ms (green) or 200 ms (blue) comparison stimulus. (B) Performance when the duration of the comparison stimulus varied. Color scheme as in panel (A). (C) Maximum slope of psychometric function for different comparison stimulus durations. The thin gray lines are fit line on data points from each individual subject. Only one out of nine human subjects showed a significant increase in the slope as a function of stimulus duration whereas all rats showed the effect. (D) Inflection point in psychometric curve, measured for different stimulus durations for *all* subjects. Gray lines are fit lines on data points from individual subjects and the thick black line is the fit line on the average data. All except one human subject showed significant shift in inflection point as a function of stimulus duration whereas only one rat subject showed such a shift. (E) The point of subjective equality (PSE) shifted in humans (left) as a function of stimulus duration but did not in rats (right).

Subject number	***p* value of slope.** Null hypothesis: stimulus duration does *not* affect psychometric curve slope.	***p* value of point of subjective equality (PSE).** Null hypothesis: stimulus duration does *not* affect psychometric curve PSE.
Rat subjects		
arr10	<0.001	0.071
ar11	0.001	0.002
ar12	<0.001	0.465
ar14	0.002	0.141
Human subjects		
1	0.367	0.148
2	0.629	0.009
3	0.008	<0.001
4	0.5	<0.001
5	0.91	<0.001
6	0.507	0.024
7	0.655	0
8	0.366	0.05
9	0.750	0

Table 3.1. Analysis of psychometric curves. The table gives the single-subject probability of the null hypothesis that stimulus duration does not affect curve slope (column 2) or point of subjective equality (column 3).

3.4 Working memory

The preceding section demonstrated that rats have vibration perception capacities qualitatively similar to that of humans. If we take psychometric curve slope as a measure of acuity, a typical human subject is superior to a rat subject; still, among the sample of 5 trained rats, the best one outperformed some human subjects. Thus, the ranges of performance are overlapping.

The design of our experiments allowed us to compare the capacities of humans and rats not only in the acuity of judging a noisy vibration, but also in the holding sensory information in working memory. The first question to be answered is – Are rats capable of forming tactile working memories? Other forms of short term memory are readily apparent in rodents. For example, the Morris water maze (Morris 1984) demonstrates spatial working memory. Rats can find a submerged platform in a pool and, after removal from the pool, can rapidly target the same platform on the next trial. Position must have been stored in memory. If the platform assumes a new position in the next set of trials, rats can quickly store the new information; thus, platform position is kept in working memory rather than a permanent store (it is not a reference memory).

But can rats acquire a signal that is essentially some value along a graded dimension, hold that signal, and then compare the value to a successive stimulus? Nonhuman primates can compare successive fingertip vibrations as a working memory task (Romo, Hernandez et al. 2002) (Romo, Hernandez et al. 2002) , but the neuronal network involved in the task involves a set of cortical regions, including supplementary motor cortex, dorsolateral and ventromedial prefrontal cortex, that may not even exist in the smaller and simpler rodent brain. Consistent with the notion that rats may not be able to perform graded working memory tasks were personal communications from several colleagues who attempted for many years without success to adapt the delayed comparison task designed by Romo and Colleagues to rats. Still, having found that rats can compare stimuli presented in succession, it seemed worth the effort to pursue tactile working memory; if the simpler rodent neocortex can accomplish short term information storage without extensive prefrontal cortical networks, the mechanisms for an efficient process in a simpler brain might be accessible.

The earlier section presented experiments in which the delay between base and comparison stimuli was 800 ms; this might already qualify as a brief working memory. Now we present results in which the delay period was extended to 10 s. To ensure that rats compared two stimuli, each base stimulus was followed by either a higher or a lower comparison stimulus; similarly, each comparison was preceded by either a higher or a lower base. This forced rats to “attend to” and compare the two stimuli – applying a threshold to just one stimulus would lead to chance-level performance. The stimulus design is fully described in Methods. The main result is given in Figure 3.4. Rats were able to perform well above chance with inter-stimulus delays up to 10 seconds, the longest tested interval.

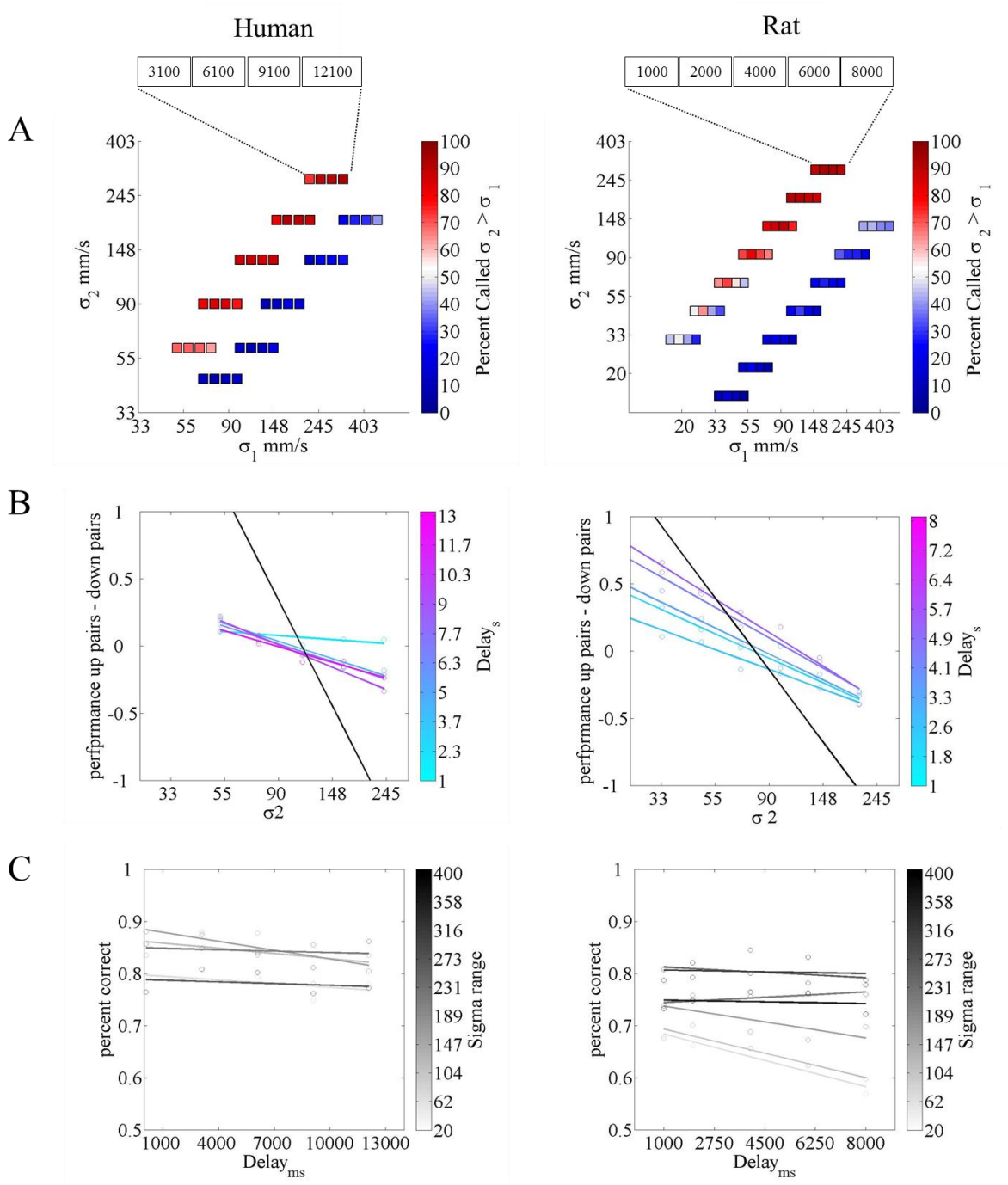


Figure 3.4. Effect of delay duration on performance. (A) Performance averaged over all 13 human subjects (left) and 3 rat subjects (right). The color code shows the percent called $2 > \sigma 1$. An ideal performer would show all pairs above diagonal line deep red and all pairs bellow diagonal line deep blue. The inset shows the delay duration for each box arranged horizontally for each $\sigma 1, \sigma 2$ pair. (B) To quantify the effect of increasing delay interval in our comparison task, we calculated the difference in performance for a given pair of stimuli with equal Standard Deviation Difference Index, defined as (σ2- σ1) / (σ2- σ1), and opposite $\sigma 1$ and $\sigma 2$ (e.g. performance of stim1($\sigma 1 = 31.2, \sigma 2 = 15$) – stime2($\sigma 1 = 15, \sigma 2 = 31.2$)). This is a test of the effect of order. For a performer who applies the comparison rule ideally, this measure would give a value of 0 for delay intervals short enough to remember the first stimulus. As the delay increases, the memory of the first stimulus would decay and the performer would report given only the second stimulus. The black line in this figure shows a hypothetical subject who does not apply the comparison rule, and instead categorizes the

second stimulus. Rats and humans perform equally well for short delays. Longer delays instead result in enhanced Contraction Bias ((Ashourian and Loewenstein 2011)in rats, as the subjects tend to more frequently judge as $\sigma 1 > \sigma 2$ for small sigma values (positive values of Standard Deviation Difference Index for (σ <60) and $\sigma 2 > \sigma 1$ for large ones (negative values of Difference Index for (σ >60)). (C) Average performance of each pair of stimuli, with equal Standard Deviation Index and opposite $\sigma 1$ and $\sigma 2$, is plotted for different delay intervals. Different lines shows different values of $\sigma 1$.

3.5 Psychophysical reverse correlation

Sensory stimuli normally consist of some mixture of physical features and in naturalistic or stochastic stimuli, the combination of features can be particularly complex. When the task of the subject is to make some decision about a stimulus, that judgment can be made using a single feature or some combination of features Because neurons encode stimulus features in a heterogeneous manner (Maravall, Petersen et al. 2007), the investigator can learn about the neuronal processing at work during the task by determining which stimulus features most systematically influence the subjects' decisions.

The general approach to identify the stimulus features that influence judgment is to correlate the choices made across a series of trial with the stimulus variability in those same trials. If a specific feature affects choice, then the distribution of values of that feature will differ between the two sets of trials corresponding to two opposite behavioral choices. This approach can be termed psychophysical reverse correlation ((Nienborg and Cumming 2007), (Neri and Levi 2006)), and its goal is to uncover the statistical relationships between random perturbations (i.e., "noise") within the stimulus and the subject's percept. From this method, one aims to determine which features are felt by the subject and how they are combined.

Thanks to the stochastic structure of the stimuli used in this task, an extensive set of stimulus features was present. We looked for correlation between the variance of each feature and the choice of subjects. Stimuli in our experiment were characterized by trial-by-trial variability over different features, some independent of each other and some correlated. For instance, due to the intrinsically "noisy" property of the stimulus, in the condition where $\sigma 1 = \sigma 2$ there were trials in which the mean absolute value of velocity in the base stimulus was slightly *larger* than that in the target stimulus while the mean value of acceleration in the base stimulus was slightly *smaller* than that in the target stimulus; mean velocity and mean acceleration could be decorrelated.

Inter-trial variations in stimulus duration offer an opportunity for further exploration of the neuronal processing underlying perception. Are the values of stimulus features integrated by a time-averaging process or by summation over time? For a given value of σ, if the percept derives from a time-averaging process then the perceptual judgment of a subject will not depend on stimulus duration. If the percept derives from a summation process, then trials in which the subject makes the two opposing judgments will be found to differ in stimulus duration. Details on the reverse correlation procedure are given in Methods and a brief

outline is presented here. To determine whether a selected feature influenced the subject's choice:

(i) According to the subject's decision on a given trial, one stimulus was labeled as "σ perceived as larger" and the other stimulus "σ perceived as smaller".

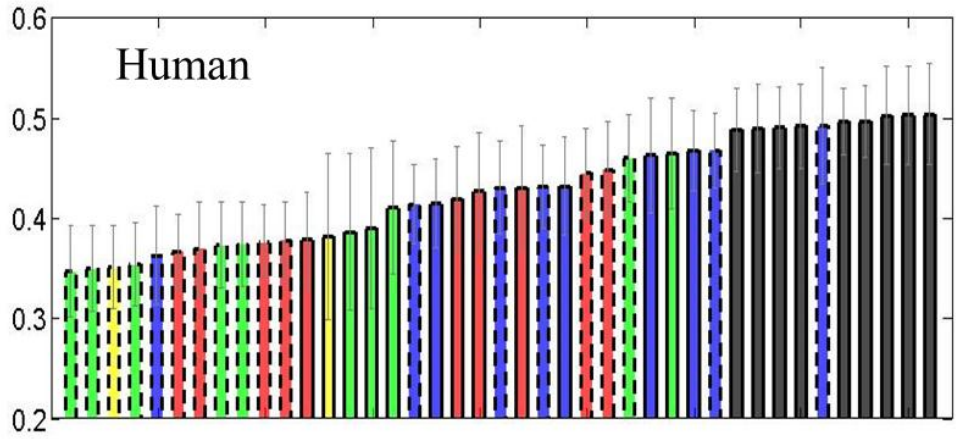
(ii) For each feature of interest, the value of that feature on the "smaller" trial was subtracted from the value on the "larger" trial.

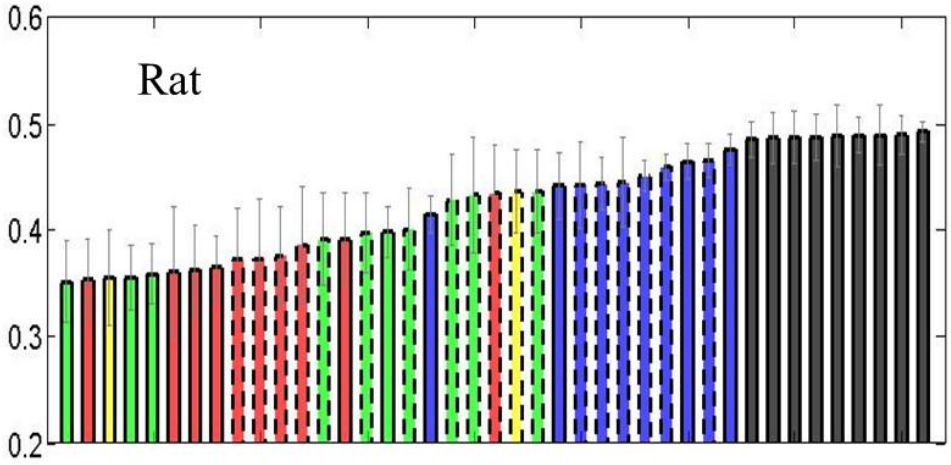
(iii) If the resulting distribution of values had a central tendency >0, we posit that higher values of that feature led to a percept of *higher* estimate of σ. In contrast, if the resulting distribution of values had a central tendency <0, we posit that higher values of that feature led to a percept of *lower* estimate of σ.

(iv) Based on (iii), we made a prediction about the subject's choice on each trial. For instance, if the "perceived larger" minus "perceived smaller" distribution is positive, we predict that on a trial in which the feature of interest is greater in the base stimulus than in the comparison stimulus, the subject will have judged σ1 as greater than σ2. We refer to the predicted choice of the subject as the "ideal observer" output on that trial.

(v) The steps listed above were applied only to trials in which the nominal difference index (σ2- σ1) / (σ2- σ1) was less than or equal to 0.1. (If the stimuli differ by a larger amount, then all individual features differ by the same sign so the reverse correlation becomes uninformative.)

From the procedure listed, we formulated a sequence of predicted choices of the ideal observer, which can be compared to the actual choice of the subject on the same sequence of trials. For each feature, we computed the normalized Hamming distance (equation 2.4) between choice of subjects and an ideal observer, which measures the minimum number of substitutions required to change one string (e.g. choice of animal) into the other (ideal observer response based on the single-trial values of that feature), or the number of errors that transformed one string into the other (see Methods). A low normalized Hamming distance (close to 0) suggests that the considered feature has a relatively large weight in the participant's decision inasmuch as the values of that feature successfully predict choices. Conversely, a large normalized Hamming distance (close to 0.5) indicates that the considered feature exerts little or no influence on the participant's decision. Figure 3.5 shows the features sorted by their normalized Hamming distance values for human (upper plot) and rats (lower plot). Different colors represent different feature types; for instance all features related to "speed" (e.g. number of speed outliers, value of the speed outliers or summation of speed over time) are in yellow. Derivation of different features is presented in Table 2.2. The style of boundary of each bar indicates whether the feature is assessed per unit time (solid boundary: rate) or summated over time count (dashed boundary: sum). The first four features used by human and rats subjects are shown in Table 3.2.





Border line style	
– – – –	Summed up or count over samples
———	Normalized over time or samples

Filled color	feature
	position
	speed
	velocity
	acceleration
	Control

Figure 3.5. Psychophysical choice probability analysis. Normalized Hamming distance for all examined stimulus features.

As a test of the method, control features were selected on the intuition that they would have no effect on choice. The test confirmed that such features gave Hamming values of 0.5.

As predicted by the study of stimulus duration-based bias (Figures 3.3-3.4), human subjects tended to summate features over time whereas rats tended to normalize the feature value over time. Features calculated on "velocity" and "speed" values are the best candidates to modulate the percept both for human and rat subjects. Features calculated on "position" are among the least informative features for rats.

This shows that human subjects rely on velocity and speed features and moreover they tend to summate the value of a given feature and not normalize it over time. The psychophysical reverse correlation thus offers a parsimonious explanation for why human subjects, but not rats, tended to overestimate target stimulus σ when its duration was greater than that of the base stimulus. Conversely, rats are able to normalize features over time, which results in *improved* estimation of the stimulus for longer stimulus duration – a steeper slope in the psychometric function – rather than an overestimate of σ.

Human		Rat	
1	Summation of positive velocity outlier events	1	Velocity standard deviation
2	Summation of negative velocity outlier events	2	Summation of negative acceleration outlier events per duration
3	Summation of velocity	3	Summation of positive velocity outlier events per duration
4	Summation of Speed	4	Speed standard deviation

Table 3.2 The four highest ranked features for human subjects and rats.

N°	Feature name	Rank of the feature in Rats	Rank of the feature in Human
1	Position standard deviation	18th	17th
2	Velocity standard deviation	1st	16th
3	Acceleration standard deviation	7th	21st
4	Speed standard deviation	4th	13th
5	Summation of Speed	21st	3rd
6	N° of positive velocity outlier events	19th	8th
7	N° of positive velocity outlier events per duration	16th	29th
8	Summation of positive velocity outlier events	17th	1st
9	Summation of positive velocity outlier events per duration	3rd	14th
10	N° of negative velocity outlier events	23rd	9th
11	N° of negative velocity outlier events per duration	15th	27th
12	Summation of negative velocity outlier events	13th	4th
13	Summation of negative velocity outlier events per duration	5th	15th
14	N° of positive acceleration outlier events	12th	10th
15	N° of positive acceleration outlier events per duration	11th	25th
16	Summation of positive acceleration outlier events	10th	11th
17	Summation of positive acceleration outlier events per duration	6th	19th
18	N° of negative acceleration outlier events	14th	7th
19	N° of negative acceleration outlier events per duration	8th	26th
20	Summation of negative acceleration outlier events	9th	12th
21	Summation of negative acceleration outlier events per duration	2nd	22nd
22	N° of positive position outlier events	26th	23rd
23	N° of positive position outlier events per duration	28th	36th
24	Summation of positive position outlier events	27th	18th
25	Summation of positive position outlier events per duration	25th	28th
26	N° of negative position outlier events	30th	20th

27	N^{o} of negative position outlier events per duration	29th	30th
28	Summation of negative position outlier events	31st	24th
29	Summation of negative position outlier events per duration	32nd	31st
30	Summation of position	35th	37th
31	Summation of velocity	39th	34th
32	Summation of acceleration	33rd	33rd
33	Summation of position per duration	36th	38th
34	Summation of velocity per duration	38th	32nd
35	Summation of acceleration per duration	34th	35th
36	Summation of absolute position	24th	6th
37	Summation of speed	22nd	2nd
38	Summation of absolute acceleration	20th	5th
39	Mean position	40th	40th
40	Mean velocity	41st	42nd
41	Mean acceleration	42nd	41st

Table 3.3 Complete rank of features for rat and human subjects

4. Neuronal analysis methods

4.1 Surgery

After rats reached a performance of more than 75% correct, they were anaesthetized with 2-3% Isoflurane in oxygen delivered through a snout mask. 5 small screws were fixed in the skull as a support for dental cement. One of the screws served as a ground electrode. A craniotomy was then made over barrel cortex, centered 2.8 mm posterior to bregma and 5.8 mm lateral to the midline. Dura mater was carefully removed over the entire craniotomy using a small syringe needle. The remaining pia mater, even if usually considered as not resistant to penetration, nevertheless presents a challenge to the entry of the microelectrode arrays. This resistance leads to deforming (dimpling) of the brain at the moment of penetration. To minimize brain dimpling, cyanoacrylate adhesive was applied directly to the pial surface bordering the edge of the cranial opening. This procedure fastens the top layer of the brain, the pia mater, to the overlying bone and the resulting surface tension prevents the brain from depressing under the advancing electrodes. With the brain anchored to bone, the 16 electrode tungsten array (Tucker-Davis Technologies) was inserted by slowly advancing a Narashige micromanipulator. After inserting the array, the remaining exposed cortex was covered with biocompatible silicon (KwikSil; World Precision Instruments). Rats were given the antibiotic enrofloxacin (Baytril; 5 mg/kg delivered through the water bottle) for a week after surgery. During this recovery time, they had unlimited access to water and food. Recording sessions in the apparatus began thereafter.

4.2 Electrophysiological recordings

The multielectrode array (Tucker-Davis Technologies) was comprised of 16 tungsten wires of 50-um wire diameter and impedance of 20 kΩ, at 1 KHz, measured in saline. In vivo impedance is higher, around 150-200 kΩ (Prasad and Sanchez 2012). The array was slowly advanced and then fixed at a depth of about 1000 μm, where it became possible to distinguish action potential waveforms evoked by manual whisker stimulation. The depth of the recording sites, together with the small 1–2-whisker receptive fields (usually E2-E3 and D2-D3), are consistent with an electrode tip position in layer 4. However our analyses and conclusions do not depend on the precise laminar localization of the neurons. Neuronal data were acquired using an RZ2 amplifier (Tucker-Davis Technologies). The continuous signal was amplified by a factor of 1,000–5,000, bandpass filtered between 300 Hz and 6 kHz, and digitized at 24 kHz. Spike detection and sorting were performed offline using clustering algorithms (UltraMegaSort2000 written by Hill DN, Mehta SB and Kleinfeld D). Most electrodes yielded a multiunit neuronal cluster. In total, we identified 274 multiunit clusters, out of which only the 125 neuronal clusters with stable waveform and firing rate over the course of a session were considered in the analysis.

4.3 Analysis of neuronal response

Our analysis was focused on recordings made during the condition used to compute psychometric functions – that is, those sets of trials where the base stimulus velocity variance was held constant and the comparison stimulus velocity variance distributed along a range of values. The question we focused on was: Through what coding scheme, and with what accuracy, can we explain the perceptual performance of the rat based on the activity of neurons in barrel cortex?

The results presented are a first step in understanding sensory cortical activity during vibration working memory, but a larger data set will be required for publication. Here, analysis was performed on recordings from 5 sessions from 1 rat. In one other rat the neuronal signals were not satisfactory for analysis. The criteria for including neurons in the analysis included:

- quality of action potential waveform as compared to electrical noise
- response of at least 1 spike per stimulus
- stable firing rate over the course of a session.

Raster plots and PSTHs

We carried out a number of steps for illustrative purposes, though they are not intended as a statistical characterization of activity. First, we illustrated the spiking activity of each cell cluster as a raster plot, namely, a sequence of events (action potential times, illustrates as dots, with 1 ms resolution) distributed along the time course of the trial. Next, we computed the peristimulus time histogram (PSTH) by averaging the spike counts within stimulus-aligned time bins of 1 ms across trials. Next, we calculated firing rate histograms for each stimulus condition (comparison stimulus value) separately and smoothed it with a Gaussian kernel (standard deviation, 50 ms). In the next section we summarize an initial statistical analysis of stimulus coding.

Dependence of neuronal activity on stimulus parameters, and relation to behavior

Measures of the relationship between neuronal stimulus coding and the animal's behavior are known as neurometric functions. In the set of trials analyzed here, the base stimulus velocity variance was kept constant at 80 mm/s (see Figure 2.4B for stimulus set). To quantify the response of each neuron as a function of stimulus velocity variance (σ), we calculated the firing of that neuron during the presentation of the comparison stimulus. Then we computed the best linear fit to firing rate as a function of σ, and measured the slope of the linear fit. To find whether the slope was significantly different from zero, we shuffled the σ value among trials. Then we found the best linear fit under the shuffled condition. We repeated the shuffling procedure 1,000 times to find the expected distribution of slopes if σ had no true effect on firing rate. The null hypothesis, then, is that the actual slope of linear fit of a given neuron comes from the null distribution. If the observed value of the slope exceeded the

expected value by 3 standard deviations then we reject the null hypothesis and conclude that the neuron's firing rate varied according to the value of σ. Note that this procedure does not specify whether a significant slope was positive or negative. Our hypothesis was that slopes would be positive, reflecting increased firing rate with increased σ.

Our main idea is that the rat used the activity of the sampled neurons, and many neurons similar to those sampled, in order to judge the stimuli. To evaluate this idea, we compared the neuronal response during the second stimulus, *r2*, in spikes per stimulus with the neuronal response during the base stimulus, *r1*. The model is that the rat judges $\sigma 2 > \sigma 1$ if $r2 > r1$. The neurometric function is then plotted as the percent of trials in which $r2 > r1$ as a function of the standard deviation index (SDI equation2.1, introduced under Behavioral Methods).

We fit the response plot of a given neuron with the same 4 parameter logistic function used for the behavioral analysis (see Behavioral Methods). The slope of this function could be used as a measure of the average performance that could be supported by decoding firing rate of each neuron. For many neurons the fitting procedure failed to pass the criteria of "goodness of fit" and this led us to use the actual values of neurometric and keep the neurometric curve only for illustration purposes. Therefore, to correlate the neuronal activity with the performance we used values of the performance of each neuron taken from neurometric function for different SDI and compared it with actual performance of the animal.

It will be seen that the neurometric functions of many neurons had a rising sigmoid shape; however the highest performance of ideal observer on the single neuronal clusters was much lower than the actual behavior

Generalized Linear Model

Understanding how neuronal responses are correlated with behavior is a fundamental question in neuroscience. It is of a great importance to know whether the information carried by individual neurons is sufficient to explain the subject's performance, or else a population code must be brought to bear. There have been a few studies showing that "single neurons are as good as the whole brain;" in other words, an ideal observer could perform the task as well as the subject by only relying on the information of an informative single neuron (Parker and Newsome 1998, Luna *et al.,* 2005). In these studies the responses of single cells were averaged over trials to enhance the signal to noise ratio and reduce the effect of neuronal variability. However, in the majority of cases, single neurons are much less informative about the task than would be required to account for observed behavior and it is more plausible that

the brain uses the information of large groups of neurons to encode stimuli – a population code (Pouget, Dayan et al. 2000) *(Georgopoulos, Schwartz et al. 1986)*).

In our experiment, some recording sessions included neuronal activity sampled from sets of 17 to 38 neuronal clusters, allowing us to assess the information carried by neuronal populations. In order to understand how the population of neurons in barrel cortex is correlated with behavior, we used a generalized linear model (GLM). This model allows linear combinations of neuron responses (firing rate or spike count), even when the neurons possess different temporal response distributions The linear combination is then mapped into desired output variables (in our case psychometric values) through a "link" function. We have used the "iteratively reweighted least squares" algorithm to find the maximum likelihood estimates of output variables. In particular, we have used comparison binomial GLM as follow:
Equation 4.1:

$$\lambda = \frac{1}{1 + \exp\left(-1 * (\beta_0 + \beta_1 \Delta r_1 + \beta_2 \Delta r_2 + \beta_3 \Delta r_3 + \cdots + \beta_j \Delta r_j)\right)}$$

where λ is the predicted outcome, in this case the probability of choosing $\sigma 2 > \sigma 1$ (note that λ is 1 for $\sigma 2 > \sigma 1$ and 0 for $\sigma 2 < \sigma 1$) , $\Delta r_{1\text{-}j}$ is difference in neuronal responses to the base and comparison stimuli, and $\beta_{0\text{-}j}$are coefficients determining the amount each neuron contributes in predicting output behavior (sometimes referred to as the neuron's "weight"). In our study we solved the GLM using two different behavioral outputs: either the ideal behavioral outcome which is 1 for $\sigma 2 > \sigma 1$ and 0 for $\sigma 2 < \sigma 1$, or the psychometric curve extracted from subject behavioral response.

5. Results of neurophysiological investigation

This chapter presents an exploration of how the neuronal activity of rat barrel cortex might encode noisy whisker motion, and how the coding might underlie behavioral performance. We carried out extracellular recordings of action potentials while the animal performed the delayed comparison task described in Chapters 2-3. Only data related to the construction of the psychometric function are included. The stimulus set is illustrated below, as reproduced from Figure 2.4B.

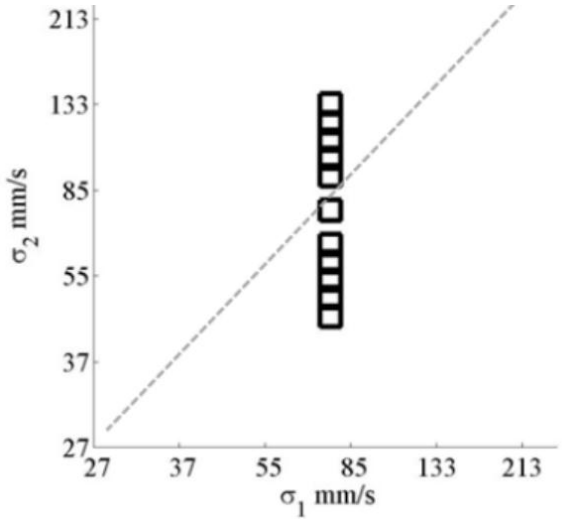


Figure 5.1. Stimulus set. The base stimulus was fixed and comparison stimulus varied across trials. Neuronal activity was recorded while the psychometric function was mapped.

The base and comparison stimuli both lasted 400 ms, with an 800 ms delay inserted in-between. The rat triggered the start of trial by placing its snout in the nose poke. The rat was required to remain immobile in the nose poke for at least 800 ms in order for the stimuli to be delivered. The "go" signal was sounded 500 ms after the conclusion of the comparison stimulus. This trial structure yielded 5 discrete intervals of interest: (i) pre-stimulus period, (ii) base stimulus, (iii) delay interval, (iv) comparison stimulus, (v) post-stimulus period.
During recording sessions we increased the pre- and post-stimulus periods compared to the purely behavioral study (Chapter 3) as a means to separate the neuronal activity during the stimulus representation from any other touch related events that are unrelated to the stimulus (e.g. entering and withdrawing from the stimulus delivery port would trigger neuronal response in the barrel cortex). If the stimulus presentation were not well separated from other events, it would be difficult to infer the neuronal activity related to stimulus coding.

Most barrel cortex neurons respond strongly upon stimulation of one or two principal whiskers (Petersen and Diamond 2000). In the current study we report the recorded activity from neurons with principal whisker E1, E2, D1 and D2. The shape of the plate attached to the vibrator and the sticky tape position was modified in order to stimulate the

aforementioned whiskers and to avoid as much as possible stimulating the rest of the whisker pad. No whisker trimming was performed and all whiskers were kept intact. Under these experimental conditions, it must be taken into account that on a number of trials, the rat might have collected stimulus information through whiskers projecting to cortical columns we did *not* record from, and *not* through whiskers projecting to cortical columns we did record from. We were not able to identify which whiskers contacted the plate on each trial. An implication is that the activity we recorded is a conservative estimate of the true stimulus representation because it may include firing from trials when the intended whiskers were not vibrated.

In total 125 neuronal clusters from 5 recording sessions were included in this study (Table 5.1). Out of these, 43 showed significant σ coding (35%). All 43 neurons with significant σ coding exhibited the expected, positive slope. An example of this type of neuron is shown in Figure 5.2.

We also employed signal detection theory to quantify the sensitivity of a given neuron and its correlation with the actual behavior of the animal. The probability that an ideal observer could report the comparison stimulus σ as larger (or smaller) than that of the base stimulus, given the information coming from a single cluster, was plotted as a function of stimulus difference (standard deviation index, SDI euqation2.1). This probability function is referred to as the neurometric function. Then the sigmoid parameters extracted from the neurometric function (see Table 5.1) were compared to those obtained from the psychometric function, as described in Chapters 2-3. The model is that the rat judges $\sigma 2 > \sigma 1$ if $r2 > r1$. The neurometric function is then plotted as the percent of trials in which $r2 > r1$ as a function of the standard deviation index. We fit the response plot of a given neuron with the same 4 parameter logistic function used for the behavioral analysis. For many neurons the fitting procedure failed to pass the criteria of "goodness of fit" and this led us to use the actual values of neurometric and keep the neurometric curve only for illustration purposes. The failure of fitting procedure could be due to the high amount of variability in neuronal response or the fact that the performance of the ideal observer based on neuronal response could not be explained and modeled using a sigmoidal curve. Therefore, to correlate the neuronal activity with the performance we used actual values of the performance for different SDIs. The highest performance of the ideal observer based on single neuronal clusters was much lower than the actual behavior (Table 5.1).

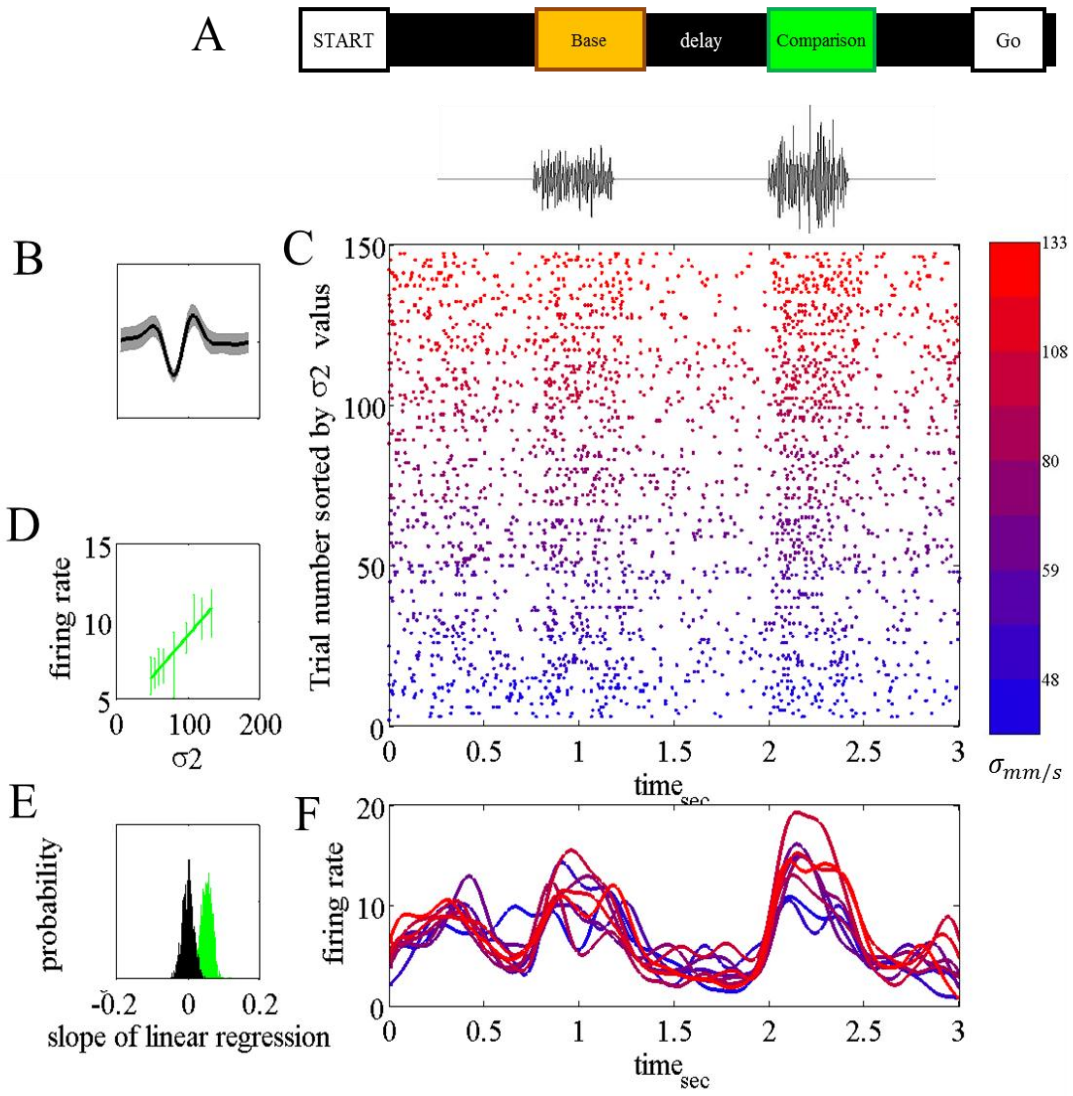


Figure 5.2. Response of a single neuron cluster. (A) Structure of a trial used in recording sessions. An example of base and comparison stimuli is depicted below. (B) Average waveforms of all spikes for this neuronal cluster are shown. Gray shadow is standard deviation over the average waveforms (C) Raster plot for all trials sorted by the σ value of the comparison stimulus. Different comparison σ's are coded by the color scale. Note that from start of the trial until the delivery of the base stimulus there was a fixed delay of 800 ms. (D) Average firing rate as a function of comparison stimulus σ. Error bars are standard error over trials. Solid line shows the best linear fit on firing rate and its slope is $0.06\ \frac{\Delta(firing\ rate)}{\Delta\sigma 2}$ (E) Distribution of slopes of the best linear fit for the same neuron. The distribution depicted in green shows the slope of the best linear fit on resampled data using bootstrap method. The distribution shown in black is the slope of the best linear fit on data coming from shuffled σ values. (F) Peri-stimulus time histogram of action potentials for different comparison σ.

Date	Number of clusters	Number of clusters with significant sigma coding ($p<0.01$)	Average percent correct from neurometric	Maximum percent correct from neurometric	Average percent correct from GLM	True percent correct of the rat
27-05-2012	38	5	0.50	0.56	0.60	0.90
2-06-2012	28	10	0.54	0.61	0.65	0.87
06-06-2012	18	12	0.56	0.59	0.67	0.89
04-06-2012	24	14	0.56	0.60	0.71	0.86
08-06-2012	17	2	0.49	0.57	0.60	0.88
Total/Average	125/5=25	43/5~=8-9	0.53	0.59	0.65	0.88

Table 5.1 Analysis of barrel cortex neuronal activity: 5 recording sessions with a total of 125 neuronal clusters. 35 percent of neurons showed significant sigma coding. Percent correct from neurometrics was calculated by assuming that the ideal observer of an individual neuron selects $\sigma 2 > \sigma 1$ whenever r2>r1.

The fact that not even a single neuronal cluster could come close to supporting the actual performance of the animal could be due to different reasons:

1. Perhaps barrel cortex is not part of the processing stream involved in this task. The neurons required to encode the stimuli might be in other parts of the brain, but not here. We think this explanation is unlikely. Even though in a recent study it has been shown that barrel cortex is required for the detection and discrimination of tactile stimuli in rats (Miyashita and Feldman 2012), in the future ablation experiments will be necessary

2. There are neurons in barrel cortex with higher sensitivity and less variability over trials than those we recorded. Perhaps the rat can selectively use these more informative neurons. We do not consider this likely because the number of sampled neurons was large enough that, if expert coding neurons existed and the brain used them, we would expect to find some indication.

3. Even if we optimized the position and the shape of the plate to stimulate only the principal whiskers of the sampled neurons, it is possible that other whiskers were also stimulated and neurons responding to those whiskers were responsible for the sensory representation. We consider this not likely to be the best explanation. We restricted the sticky tape to the part of the plate in contact with the principal whiskers of the neurons reported here. Although we cannot guarantee that other whiskers were not stimulated, it must be noted that when we removed the sticky tape the performance of animal dropped to chance level. This suggests that the animal was mainly relying on principal whiskers that we were recording from.

4. We may have applied the wrong decoding model to neuronal firing. Stimulus information might be encoded more robustly in a temporal code for instance.

5. The final possibility is that the information is coded by firing rate, as we suppose, but by a larger group of neurons (a population code) rather than by single neurons. Target neurons would integrate the collective activity of the barrel cortex population in order to judge the stimuli. There are many reasons to think that neurons may carry an incomplete message taken singly, but a robust message as a population. For instance, in our task, stimuli are composed of a continuous gradient of kinematic features, presented in random order (excepting for the stimulus correlation inevitable due to mechanical constraints). One neuron might encode one feature, such as absolute speed, but too sparsely to specify the stimulus on a single trial. Another neuron might encode another correlated feature, like acceleration. Another neuron might encode the same feature but with a high threshold, firing only for outlying events. In summary, the selected stimulus might not be fully encoded by individual neurons whereas an ensemble could give a more complete picture. To test this hypothesis we employed a generalized linear model (GLM)-based analysis of the neuronal population activity. In ordinary linear regression methods, it is expected that the value of output changes as a linear combination of a set of observed values. In our case the probability of calling $\sigma 2 > \sigma 1$ would result from a linear combination of the firing rates of different neurons.

The method is better elucidated by an example. Suppose under the condition that $\sigma 2 > \sigma 1$ the animal reports $\sigma 2 > \sigma 1$ on 90% of trials. If $\sigma 2$ is increased by 10% and neuronal firing rate doubles, a simple linear regression model would predict that the probability of judging $\sigma 2 > \sigma 1$ would increase to 180% or some linear function of the firing rate. But we know that the response of the subject is not linear and instead follows a sigmoidal shape. GLM addresses this issue by allowing linear combinations of neuronal responses even when the neurons possess different temporal response distributions. The linear combination is then mapped into desired output variables (in our case psychometric values) through Log-odds as a "link" function.

Our principal finding is that the performance supported by the GLM is much closer to the animal's true performance (Table 5.1). To rule out the possibility of over-fitting of the data, we divided trials into 2 subsamples. The first subsamples were used to estimate and optimize the model parameters (see Neuronal Analysis Methods); then we used the optimized parameters on test trials. To define the confidence interval for the performance of the GLM, we used bootstrap methods on the test trials 1,000 times. The result shows that the performance arising from combinations of neurons is much better than the best single neuronal cluster (Table 5.1) (Figure 5.3). This result suggests that the population code accounts better for the behavior than do single neurons. Further analysis is necessary to find the true nature of this population code and to explore alternative codes, like a timing or correlation code.

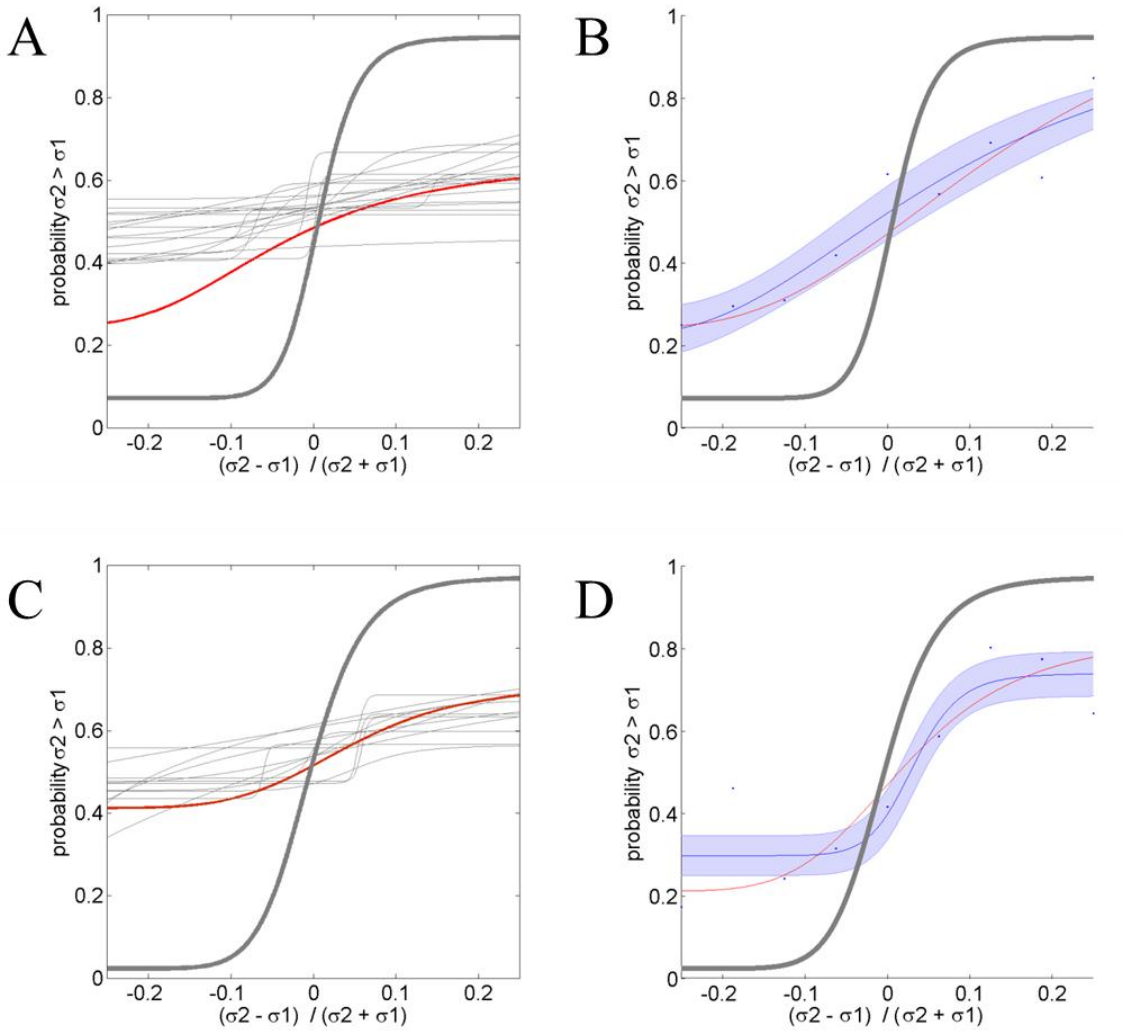


Figure 5.3. Psychometrics and neurometric curves. (A) Y axis is the percent of trials judged as σ2>σ1. Thick solid black line corresponds to the rat's performance during a single recording session and thin gray lines shows the performance of an ideal observer weighing whether σ2>σ1 based on individual neuronal clusters. The red line shows the best individual cluster. All curves depicted in this figure are sigmoid fits on data; actual data points are not shown. (B) Again, the thick solid black line corresponds to the rat's performance during the same recording session. The neurometric curves are based on the output of the GLM fit (equation 4.1) to all neuronal clusters recorded in each session. The parameters estimated on training session were applied to the same trials (red line) and resampled test trials (blue line). Blue shadow shows the standard deviation. (C and D) are the same format as A and B but for a different recording session.

6. Conclusions

We have designed an automated setup to measure rats' perception of whisker vibration. The well-controlled behavioral setup allowed us to systematically assess the behavior of the animal. Henceforth we can assess the performance of human and rats and compare their ability on the perception of noisy tactile stimuli, working memory and decision-making. Moreover simultaneous recording of neural activity of barrel cortex and behavior led us to decipher a putative neuronal code and its correlation with the judgments of the animal. Here we list three main findings:

First, we have observed that both human and rats performed the task well. As was expected, performance improved as a function of the difference between the two stimuli. Overall performance of human subjects was better than rats. Both human and rats could perform the task with long retention interval (12s for human and 10s for rats).

The unique pair design used in this task allowed us to quantify the performance in the working memory (WM) task. The traditional method for quantifying WM capacity uses the decay in performance as a function of delay. In our task the main effect of longer delay can be seen on pairs composed of σ values that are at the extremes of the σ range. As the sensory memory trace of the base stimulus dissipates over long delays, the subject relies more on comparison stimulus the drop in performance could be seen in pairs used the extreme high or low range of σ values and performance on pairs in the middle range remains intact. This new method can be used to quantify and correlate the behavioral outcome for different delay interval to neural activity during the delay interval in the area of the brain accountable for holding the information in short term memory. We have manipulated the difficulty of the task in two ways: changing the difference between the two stimuli and make them similar to each other and also increasing the retention interval. We have observed striking similarities in behavior of both rats and humans.

Second, because of the stochastic nature of the stimulus, the essential properties of the stimulus cannot be inferred accurately for short time window. The task is therefore ideal to quantify the accumulation of sensory information. By manipulating the duration of the comparison stimulus, we observed the remarkable and counter-intuitive finding that rats acquired stimulus statistics over longer durations – up to at least 600ms. In contrast, human subject did not improve with increasing stimulus duration, at least over the durations used in this experiment. Instead, humans were strongly biased by stimulus duration. Humans overestimate σ for longer stimuli and underestimate σ for shorter stimuli. One possible explanation is that the shortest stimulus duration used in our task was already long enough for human subjects to reach to the maximum performance. Another explanation is that rats and humans use different strategies to solve the task. To select between these possibilities, we devised a method of psychophysical reverse correlation from which we observed that human and rats rely predominantly on features derived from "velocity" and "speed". However, human subjects tended to summate features over time whereas rats tended to normalize the

feature value over time. This difference in strategy could explain why human subjects underestimate σ for short stimuli whereas rats did not have this bias. Since rats normalize by time, they obtain a better estimate of σ as stimulus duration increases. By analyzing the performances of an ideal observer using the features that human or rats rely on the most, we noted that the summation strategy can impair the ability to accumulate information over time. The effect of the different strategies on information accumulation is modeled in Figure 6.1

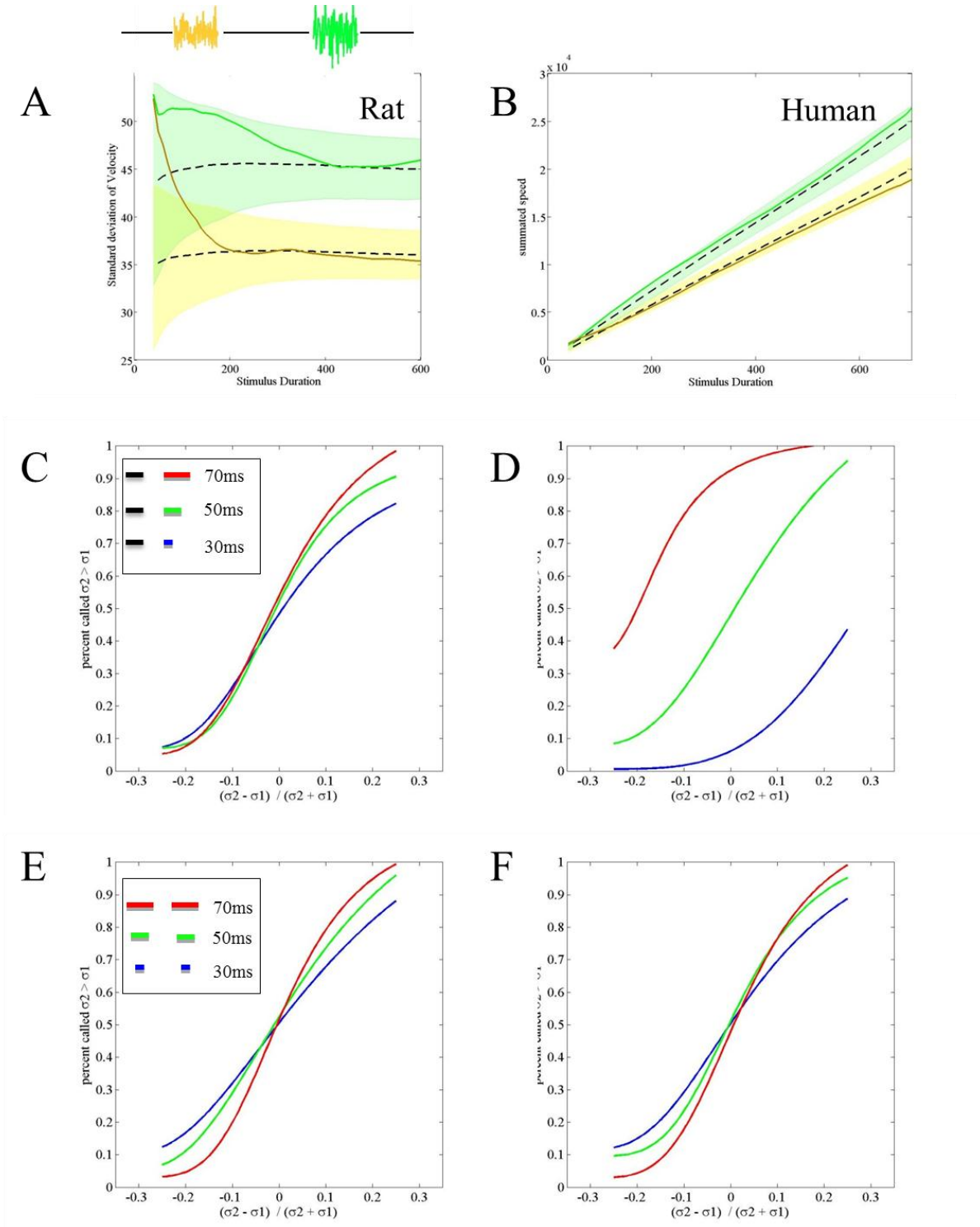


Figure 6.1 Model of the effect of stimulus duration on performance. (A) Ideal observer estimation of one of the principle features used by rats as a function of stimulus duration. The sample trial is shown above. The thick green and dark yellow lines are plots of velocity

standard deviation as a function of time for the trial shown above. The black dashed lines are the mean velocity standard deviation over many trials. The green and yellow shaded areas show the standard deviation across trials of velocity standard deviation. The standard deviation of the feature estimate decreases as a function of stimulus duration, confirming thatthe error of the estimate decreases as stimulus length increases. This would allow improved discriminability as a function of time, exactly as observed in the study of rats. (B) same as (A) for a summated feature value, which we argue is used by humans. Note that the standard deviation of the estimate increases as a function of time. (C) and (D) Ideal observer performance based on principle features used in (A) and (B). Blue green and red curves correspond to short, medium, and long stimulus duration. The duration of the base is kept constant. (E) and (F) same as (C) and (D) but for the condition that base and comparison stimulus durations vary together.

Rats normalize the principal feature value over time. By normalizing it over time the error of estimation decreases proportional to $\frac{1}{\sqrt{stimulus\ duration}}$ and the mean estimate does not change. This can be derived analytically from the *central limit theorem*. This means that longer duration leads to a more accurate estimate of σ, therefore better performance. On the other hand, human subjects summate the principal feature value. By summation, the error of estimation increases proportional to $\sqrt{stimulus\ duration}$, but also the mean estimate of the principal feature increases as a function of σ. Therefore, when comparison and base duration are equal the performance is enhanced as a function of stimulus duration. But if the duration of base and comparison are not equal, the bias of estimation of σ is more pronounced than the enhancement in performance. Even the performance can decrease compared to the situation that both stimuli durations are equal. This result demonstrates that rats use a more favorable strategy in the task used in this study compared to human subjects.

Third, we recorded neuronal activity from the barrel cortex as a well-trained rat performed the task. We observed that although 35 percent of the neurons in barrel cortex significantly coded σ, the best performance of an ideal observer based on σ-coding neurons was much lower than the actual performance of the animal. We hypothesized that the information is coded in groups of neurons.

In our task, stimuli are composed of different features that are randomly presented, and even if we systematically vary σ values of velocity, other correlated features may vary as well. Therefore, it is not accurate to estimate one stimulus by encoding only one feature at a time. Instead, combining different features over time would represent one stimulus more accurately. It has been shown that barrel cortex neurons respond to the specific kinematic features of the whisker motion. Therefore, one can hypothesis that the response of different neurons, each responding to different features, should be combined in order to code one stimulus. We employed a generalized linear model (GLM) based analysis on the population of neural activity. We observed that the predicted performance evaluated from GLM parameters (equation 4.1) are much

closer to actual animal's performance, suggesting that the combination of the information of different neurons accounts better for the behavior of the animal compared to single neurons. Further analysis is necessary to find the best population code candidate that account for this behavior.

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