Decoding the temporal evolution of a simple perceptual act

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Abstract. Most perceptual tasks require sequential steps to be carried out. This must be the case, for example, when subjects discriminate the difference in frequency between two mechanical vibrations applied sequentially to their fingertips. This perceptual task can be understood as a chain of neural operations: encoding the two consecutive stimulus frequencies, maintaining the first stimulus in working memory, comparing the second stimulus to the memory trace left by the first stimulus, and communicating the result of the comparison to the motor apparatus. Where and how in the brain are these cognitive operations executed? We addressed this problem by recording single neurons from several cortical areas while trained monkeys executed the vibrotactile discrimination task. We found that primary somatosensory cortex (S1) drives higher cortical areas where past and current sensory information are combined, such that a comparison of the two evolves into a decision. Consistent with this result, direct activation of the S1 can trigger quantifiable percepts in this task. These findings provide a fairly complete panorama of the neural dynamics that underlies the transformation of sensory information into an action and emphasize the importance of studying multiple cortical areas during the same behavioural task.

Investigations in several sensory systems have shown how neural activity represents the physical parameters of the sensory stimuli both in the periphery and central areas of the nervous system (Hubel & Wiesel 1998, Mountcastle et al 1967, Talbot et al 1968). These investigations have paved the way for new questions that are more closely related to cognitive processing. For example, where and how in the brain do the neuronal responses that encode the sensory stimuli translate into responses that encode a decision (Romo & Salinas 2001, Schall 2001)? What components of the neuronal activity evoked by a sensory stimulus are directly related to perception (Romo et al 1998, Salzman et al 1990)? These questions have been investigated in behavioural tasks where the sensory stimuli are under precise quantitative control and the subjects' psychophysical performances are quantitatively measured (Hernández et al 1997, Newsome et al 1989). One of the main challenges of this

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approach is that even the simplest cognitive tasks engage a large number of corti-
cal areas, and each one might encode the sensory information in a different way
(Romo & Salinas 2003, Romo et al 2004). Also, the sensory information might be
combined in these cortical areas with other types of stored signals representing, for
example, past experience and future actions (Hernández et al 2002, Romo et al 2002,
2004). Thus, an important issue is to decode from the neuronal activity all these
processes that might be related to perception.

Recent studies have provided new insights into this problem using a highly sim-
plified sensory discrimination task (Hernández et al 1997, Mountcastle et al 1990).
In particular, these studies have shown the neural codes that are related to sensa-
tion, working memory and decision making in this task (Romo & Salinas 2003). An
important finding is that primary somatosensory cortex (S1) drives higher cortical
areas from the parietal and frontal lobes where past and current sensory informa-
tion are combined, such that a comparison of the two evolves into a behavioral
decision (Romo & Salinas 2003). Another important finding is that quantifiable per-
cepts can be triggered by activating directly the S1 circuit that drives cortical areas
associated with perceptual decision making in this task (Romo et al 1998, 2000).
Here we discuss the evidence supporting these conjectures.

Psychophysics and neurophysiology of a simple perceptual task

We studied the extracellular activity of single cortical neurons while trained mon-
keys executed a highly simplified vibrotactile discrimination task (Fig. 1). In this
two-alternative, forced-choice task, subjects must compare the frequency of two
vibratory stimuli applied sequentially to their fingertips and then use their free hand
to push one of two response buttons to indicate which stimulus was of higher or
lower frequency. The discrimination task, although apparently simple, is designed
so that it can only be executed correctly when a minimum of neuronal operations
or cognitive steps is performed: encoding the two stimulus frequencies, maintain-
ing the first stimulus frequency (f_1) in working memory, comparing the second stim-
ulus frequency (f_2) with the memory trace of f_1, and, finally, executing a motor
response to report discrimination (Hernández et al 1997). Thus, the discrimination
task allows us to investigate a wide range of essential processes of perceptual deci-
sion making.

A simple testable hypothesis is that the sequential events associated with the
vibrotactile discrimination task are represented in the neuronal activity of a widely
distributed system, beginning in S1 and ending in the motor cortices where the
motor commands are triggered to report this cognitive operation. It is unlikely that
the ascending inputs to S1 encode the essential neuronal computations required to
solve this task. Their role could simply be to transmit a neural copy of vibrotactile
stimuli, where the stimulus location and features are safely encoded and transmit-
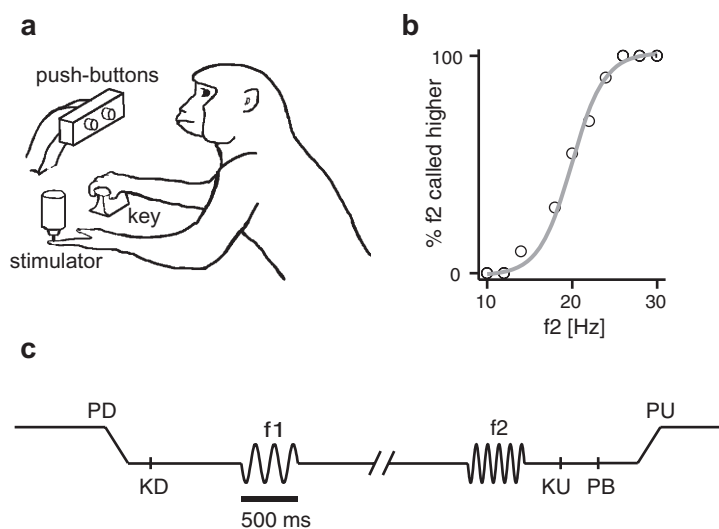
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FIG. 1.   The discrimination task. (a) Drawing of a monkey working in the discrimination task. (b) Sequence of events during discrimination trials. The mechanical probe is lowered, indenting the fingertip of one digit of the restrained hand; the monkey places its free hand on an immovable key (KD); the probe oscillates vertically at the base stimulus frequency (f1); after a delay, a second mechanical vibration is delivered at the comparison frequency (f2); the monkey releases the key (KU) and presses either a medial or a lateral push-button (PB) to indicate whether the comparison frequency was lower or higher than the base. (c) Discrimination perfomance curve, plotted as the animal's capacity to judge whether f2 is higher than f1.

ted to S1. Based on these premises, we sought the neuronal activity in several cortical areas that might be associated with the different components of the vibrotactile discrimination task. We assumed that in the neuronal responses the stimulus parameters could be quantified and interpreted according to the task demands.
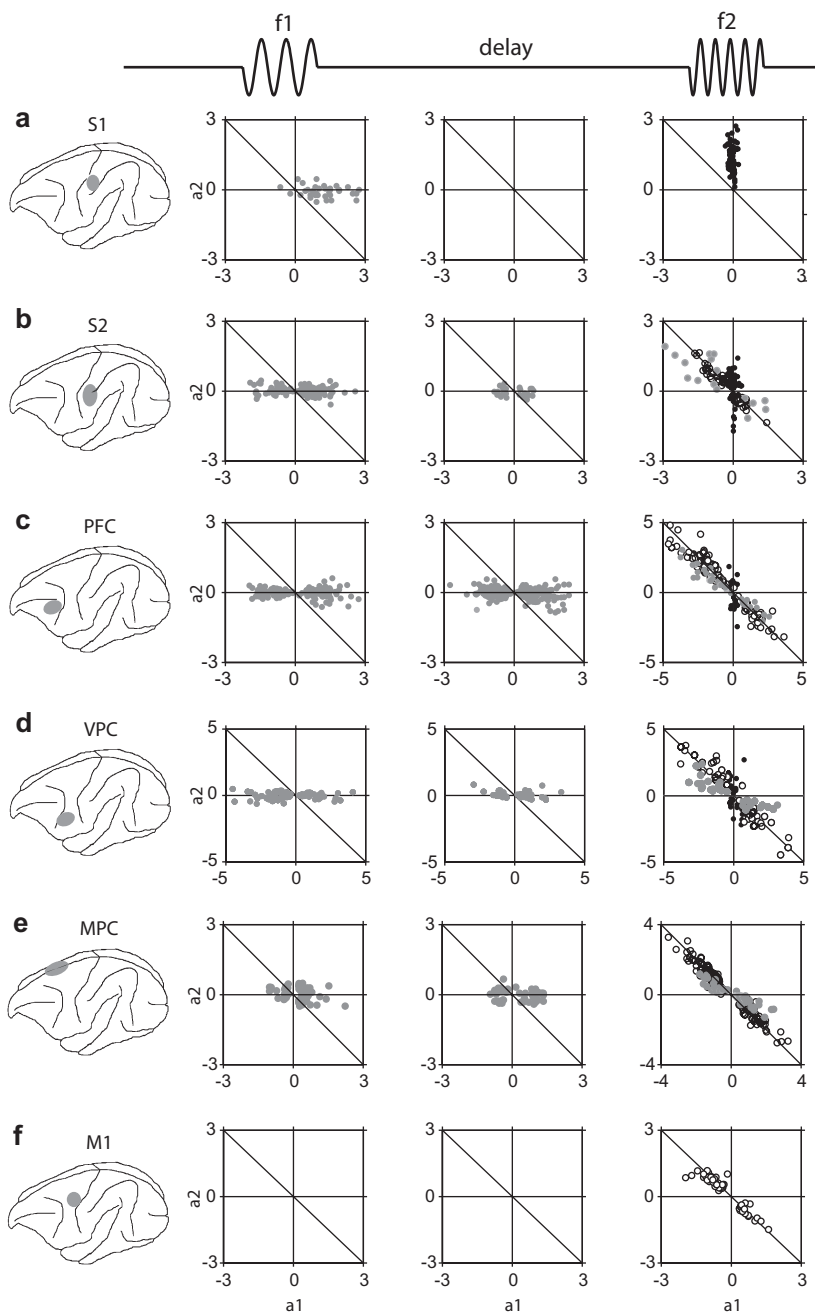
Decoding sensory processes from neuronal activity

In the vibrotactile stimulus range used here (5–50 Hz), mean responses of some S1 neurons (about 30% of the sampled population) typically increase as a monotonic function of the increasing stimulus frequency (Hernández et al 2000, Salinas et al 2000). For example, during the f_1 period, the firing rate can be approximated to a lineal function: firing rate $= a_1 \times f_1 + b$. Where a_1 and b are constants. The coefficient a_1 is the slope of the rate frequency function, and is a measure of how strongly a neuron is driven by changes in frequency (in this case f_1). To get an idea of modulation strength, a value of 1 means that the rate increases by 1 spike per second

when frequency increases by 1 Hz. This means that the firing rates of some S1 neurons usually increase with increasing stimulus frequency. Figure 2a shows slopes distributions derived from S1 responses. As it is illustrated in these plots, this analysis can be also extended to the delay period between the f_1 and f_2 periods. Clearly, none of the S1 neurons that were modulated as a function of the increasing f_1 show a modulation of this type during the delay period. This suggests that S1 neurons do not encode f_1 during the working memory component of the task.

The responses during f_2, where the comparison process takes place, could be an arbitrary linear function of both f_1 and f_2. This could be approximated by the equation: firing rate = $a_1 \times f_1 + a_2 \times f_2 + b$. Fitting this equation to neuronal responses and plotting a_2 as a function of a_1 allows a quantification of the neuron's responses dependence on the f_1 and f_2. Responses that are a function of $f_2 - f_1$ are of particular importance for our ordinal comparison task, since correct responses depend only on the sign of $f_2 - f_1$: $f_2 > f_1$ or $f_2 < f_1$. However, the analysis shows that S1 neurons do not show the comparison process during the f_2 period (Fig. 2a). They increase their firing rate as a function of the increasing f_2, suggesting that the computation between the memory referent of f_1 and the current f_2 input may occur in a central area(s) to S1.

The same analysis used to decode f_1 and f_2 can be also applied to the neuronal responses of areas central to S1. This is an important issue, because it might be possible to quantitatively show sensory information processes in these areas. For example, in secondary somatosensory cortex (S2) similar variations in firing rate are also observed as in S1, however about 40% of the neurons have negative slopes during the f1 period (Romo et al 2002, 2003, Salinas et al 2000). The firing rates of these neurons decrease as a linear function of the increasing stimulus frequency whereas the remainders have positive slopes and fire more strongly to the increasing stimulus frequency (Fig. 2b). In all areas central to S2 that have been examined so far (except for primary motor cortex [M1]) and that are active in the vibrotactile discrimination task show similar monotonic responses and similar proportions of positive and negative slopes (Fig. 2c–f). These areas are the ventral premotor cortex (VPC), prefrontal cortex (PFC) and the medial premotor cortex (MPC) (Brody et al 2003, Hernández et al 2002, Romo et al 2002, 2003, 2004). The responses seem to proceed in a serial fashion, with shorter latencies in S1 than in S2, PFC, VPC and MPC (Romo et al 2004). Whether this reflects a serial or parallel processing is not clear. There is strong evidence that S2 is driven by S1 (Pons et al 1987, Burton et al 1995), but it is not clear whether S2 drives the PFC, VPC and MPC. Some anatomical studies suggest that S2 is connected with these areas, but more studies are needed to establish whether this is so (Cipolloni & Pandya 1999, Disbrow et al 2003 Godschalk et al 1984). Thus the f_1 representation in S1 (Fig. 2a) is transformed in S2 (Fig. 2b) in a dual representation (positive and negative slopes), which is also observed in areas of the frontal lobe (Fig. 2c–f). According to these results, these

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areas of the frontal lobe that process sensory information could be also considered as parts of the somatosensory system.

Decoding memory processes from neuronal activity

One of the key features of the vibrotactile discrimination task is that it requires short-term memory storage of information about f_1. Because we did not find in S1 any trace of f_1, we wondered where and how the f_1 trace is held in the brain. The first neural correlate about this process was found in PFC (Brody et al 2003, Romo et al 1999), an area involved in working memory. The inferior convexity of the PFC contains neurons that increase their firing rate in a frequency-dependent manner during the delay period (Fig. 2c). The dependence of firing rate on f_1 is monotonic, exactly as it was observed for the f_1 periods in those areas central to S1 (S2, PFC, VPC and MPC). This mnemonic representation is not static, in the sense that the intensity of the persistent activity varies throughout the delay period. Some of the PFC neurons carry information about f_1 during the early component of the delay period, others only during the late part of the delay period, and still others persistently throughout the entire delay period. These findings suggest that in the PFC coexist distinct neuronal populations that carry information of f_1 at different times and may also indicate that the PFC circuit is composed of a chain of neurons that dynamically hold the f_1 information (Brody et al 2003, Miller et al 2003, Romo et al 1999).

FIG. 2.   Population dynamics in different cortical areas during the flutter discrimination task. Each data point represents one neuron. For each neuron, responses were fit to the equation: firing rate $= a_1 \times f_1 + a_2 \times f_2 + b$. The coefficients a_1 and a_2 were computed from responses at different times during the task. Points that fall on the $a_1 = 0$ axis represent responses that depend on f_2 only (black dots); points that fall on the $a_2 = 0$ axis represent responses that depend on f_1 only (gray dots); points that fall on the $a_2 = -a_1$ line represent responses that are functions of $f_2 - f_1$ (open circles). The data shown are significantly different from (0,0) in at least one of the epochs analysed. (a) S1 responses during the first stimulation period (f_1; left), the interstimulus period (delay; middle), and the second stimulation period (f_2; right). These neurons were active only during stimulation; most of them increased their rates with increasing frequency (positive a_1 and a_2). (b) S2 neurons respond to f_1 (left) and exhibit a modest but significant amount of delay activity (middle). Positive and negative coefficients indicate rates that increase and decrease as functions of frequency, respectively. During the initial part of f_2 (right), neurons may have significant a_1 coefficients (grey dots) or may respond exclusively to f_2 (black dots), as computed from the first 200 ms after stimulus onset. Later on, the coefficients cluster around the line $a_2 = -a_1$ (open circles dots), as computed from the last 300 ms before stimulus offset. Brain diagram shows region of approach to S2, which is hidden in the lateral sulcus. (c–f) Data from prefrontal cortex (PFC), ventral premotor cortex (VPC), medial premotor cortex (MPC) and primary motor cortex (M1) are calculated as in (b). (Modified from Hernández et al 2000, 2002, Salinas et al 2000, Romo et al 2002, 2004, and from unpublished data from Romo et al for PFC and M1.)

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An important observation regarding the working memory systems is that other cortical areas also hold information about f_1. The VPC (Fig. 2d) also encodes information about f_1 during the delay period exactly as it does the PFC (Romo et al 2004). Also some S2 neurons show a similar type of monotonic encoding (Fig. 2b and 3b), but only during the early part of the delay period, suggesting the presence of working memory signals in S2 (Salinas et al 2000). Whether these S2 neurons are the ones that drive PFC and VPC neurons during the delay period or whether the S2 neurons that respond during the f_1 periods are the ones that activate the mnemonic circuits is not known.

One wonders about this mnemonic coding scheme. Is there any distinction about the functional role of these mnemonic neurons found in these cortical areas? There are a couple of additional observations that may shed light to this question and they came from recordings in the MPC (Hernández et al 2002). First, the MPC contains neurons that encode f_1 during the late part of the delay period, just before the presentation of f_2 (Fig. 3e). Again, with similar monotonic responses and similar proportions of positive and negative slopes (Fig. 2e). Second, the dynamics of these neurons are similar to those from PFC and PVC that encode f_1 during the late part of the delay period (Brody et al 2003, Romo et al 1999, 2004). This would suggest a coding mnemonic scheme according to the task demands. Information about f_1 must be available during the f_2 period for the comparison with the f_2 input and persistent and late neurons might provide it (Fig. 3). Persistent and late neurons are therefore well positioned to compute the comparison process.

Decoding comparison processes from neuronal activity

Reaching a decision in the vibrotactile discrimination task requires the comparison between the memory trace of f_1 and the current sensory input of f_2. We sought evidence of this operation in S1, but as indicated already the activity of these neurons do not combine f_1 and f_2 during the comparison period; they encode only information of f_2. Where and how is this neuronal operation executed? A simple inspection of the neuronal activity in areas central to S1 indicated that the responses during the f_2 period are quite complex (Figs. 2 and 3). For example, some S2 neurons encoded f_2 in their firing rates similarly as for f_1 (positive and negative slopes). But, surprisingly, many S2 neurons responded differentially during the comparison $f_2 > f_1$ or $f_2 < f_1$ trails during correct discriminations (Romo et al 2002). These differential responses were even more abundant in areas of the frontal lobe (PFC, VPC, MPC and M1) examined in this task (Hernández et al 2002, Romo et al 2004). The question is whether the responses during f_2 depended on f_1, even though f_1 had been applied 3 seconds earlier, or whether they simply reflected their association with the motor responses. We ruled out the presence of a simple differential motor activity associated with the push-button presses by testing these neurons in a control
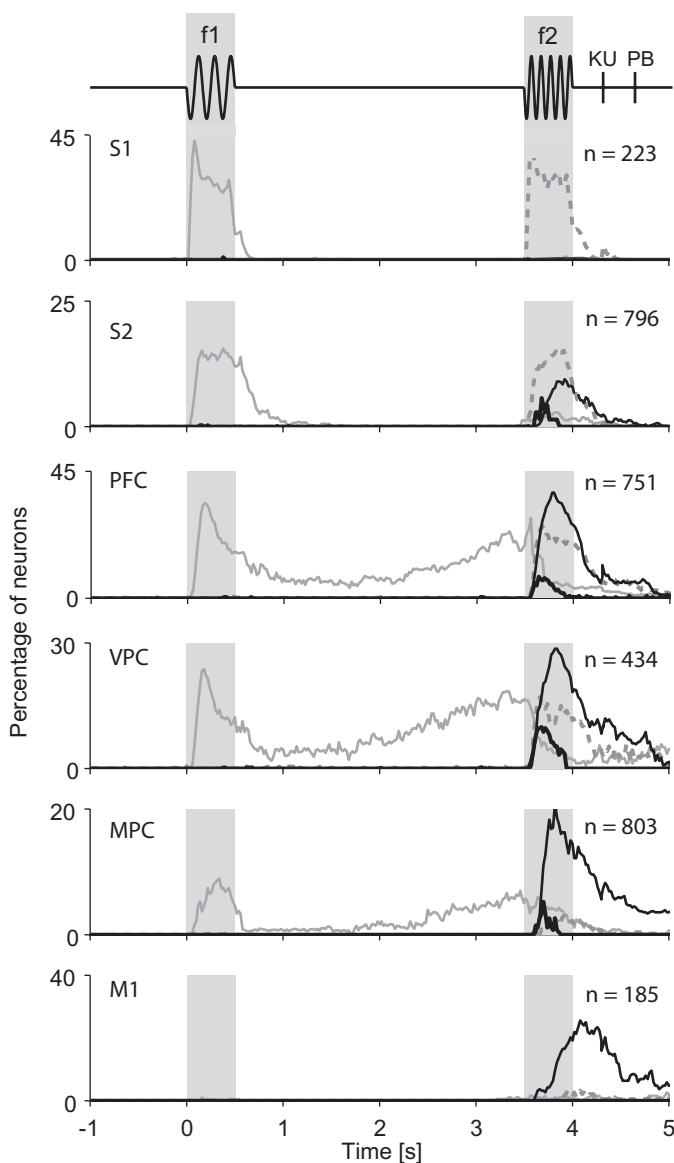
FIG. 3. Dynamics of population responses of six cortical areas during the vibrotactile discrimination task. Coefficient values of a_1 (continous grey trace), a_2 (doted grey trace), and as a function of the interaction between a_1 and a_2 (continous black traces indicate those neurons that show $f_2 > f_1$ or $f_2 < f_1$; thick black traces indicate those neurons that show the actual difference between f_2 and f_1). The responses are expressed as percentage of the total number of neurons (*n*) that had task-related responses. S1, primary somatosensory cortex; S2, secondary somatosensory cortex; PFC, prefrontal cortex; VPC, ventral premotor cortex; MPC, medial premotor cortex; M1, primary motor cortex. Original data from S1, S2, MPC, PVC and M1 were previously published (Hernández et al 2000, 2002, Romo et al 2002, 2004) and data from PFC are unpusblished results.

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task where the same vibrotactile stimuli were used, but animals had to follow a visual cue to produce the motor responses. In this condition all neurons reduced the differential activity (Fig. 4), indicating that the differential activity observed during the comparison period depends on the actual computation between f_1 and f_2 and does not reflect a purely motor response aimed to press one of the two push-buttons (Hernández et al 2002, Romo et al 2002, 2004).

If the neuronal discharges during the comparison period are the product of the interaction between f_1 and f_2, then the trace of f_1 and the current f_2 could be observed during the comparison period before the discharges indicated the motor decision responses. To further quantify these interactions between f_1 and f_2 and beyond it, we used the multivariate regression analysis described already. The analysis revealed the contributions of f_1 and f_2 during the comparison period for S2, PFC, VPC and MPC neurons (Fig. 2). This is clearly shown in the successive time windows by plotting the coefficients a_1 and a_2 and the absolute difference between the two ($a_1 = -a_2$) during the entire sequence of the vibrotactile task (Fig. 3). This allows appreciating the time dynamics of the neurons' response dependence on f_1 and f_2 for each of the cortical areas that are active during the vibrotactile discrimination
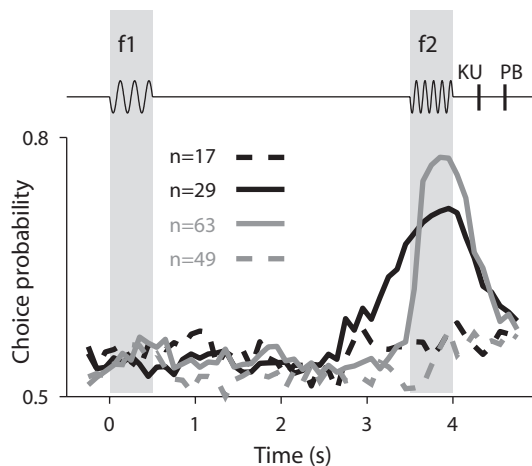

FIG. 4. Correlation between neuronal and behavioural responses. Choice probability indices for ventral premotor cortex (VPC) neurons as functions of time for three different groups of neurons. Results are averaged over (f_1, f_2) pairs. Black broken lines, responses that depended only on $f2$ during the comparison period. Black trace, neuronal responses that depended on $f1$ during the delay period and on $f_2 - f_1$ during the comparison period. Grey trace, neuronal responses that depended on $f_2 - f_1$ during the comparison period, but were not significant during the delay period between f_1 and f_2. Grey broken trace, neuronal responses that had large choice probability indices (black and grey continuous traces), but tested in a control task in which animals had to follow a visual cue to produce the motor response. (Modified from Romo et al 2004.)

task. This comparative analysis shows that the decision-making process is widely distributed through the cortex, although with various strengths across these areas (Romo et al 2004). The comparison signal evolves into a signal, which is consistent with the motor choice, but this is again stronger in some areas than in others, but it is widespread nonetheless. The resulting motor signal is also observed in M1, but M1 does not seem to participate in the sensory, memory and comparison components of the task (Fig. 3f). Also, the differential signal in M1 is considerably delayed in comparison to S2, PFC, VPC and MPC (Romo et al 2004). The results suggest that the comparison between stored and ongoing sensory information takes place in a distributed fashion. But, do these neurons predict in their activity the motor decision report?

Decoding decision processes from neuronal activity

Responses during correct trials alone do not allow us to determine to what extent the comparison dependent responses observed in S2 and frontal lobe are correlated with the sensory evaluation, or with the monkey's action choice itself (Figs. 2, 3). To answer these questions, for each neuron we sorted the responses into hits and errors and calculated a choice probability index (Green & Swets 1966, Britten et al 1996, Hernández et al 2002, Romo et al 2002, 2004). This quantified for each $f_2 - f_1$ pair whether responses during error trials were different from responses during correct trials (Fig. 4). If the responses were exclusively stimulus dependent, they should show little or no difference between error and correct trials. In contrast, if the responses were linked to the monkey's choice, then the responses should vary according to which button the monkey chose to press. In principle, this represents the probability with which an observer of a neuron's response to a given (f_1, f_2) pair would accurately predict the monkey's choice. We found that the closer a neuron's responses to correct trials were purely $f_2 - f_1$ dependence, the higher the separation between responses to correct and error trials, as quantified by a higher choice probability. We also found that the choice probability indices increased during the course of the f_2 period. This was quite evident for those neurons that had $f_2 - f_1$ responses but not for those neurons that responded to f_2 only. This tendency was observed for each area examined central to S1 (Romo et al 2004). We illustrate these processes for subgroups of VPC neurons (Fig. 4). An interesting finding was that the neuronal population that carried f_1 information during the delay period also shows large choice probability (above 0.5) values just before the comparison period (Fig. 4). We suggest that this activity is related to the working memory component of the task as opposed to the decision component of the task. If trial-by-trial variations of f_1 encoding during the working memory period correlate with trial-by-trial variations in performance, this will then be reflected in the choice probability index. The choice probability analysis shows that responses from

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S2 and the frontal cortex reflect the active comparisons between f_1 and f_2 and the motor choice that is specific to the context of the vibrotactile discrimination task.

Generating artificial percepts by cortical microstimulation

We have shown how the neuronal activity from several cortical areas is associated with the different components of the vibrotactile discrimination task. But, do these neuronal correlates actually have a direct impact in the task? Intracortical microstimulation is a powerful technique that can be used to directly prove whether the activity of localized groups of neurons is causally linked to the cognitive components of this task (Salzman et al 1990). This approach can be used to test whether the S1 representation of the stimuli is sufficient to trigger all the cognitive processes of the task (Romo et al 1998, 2000). Previous experiments had shown that the quickly adapting (QA) neurons of S1 encode the stimulus frequency both in their periodicity and firing rates (Mountcastle et al 1969). However, an analysis of the neural activity using detection theoretic analysis showed that the firing rate representation correlated with the monkey's psychophysical performance (Hernández et al 2000, Salinas et al 2000). Thus, the microstimulation approach may be useful to test which or these two codes (periodicity or firing rates) are meaningful for discrimination in this task.

Figure 5 summarizes results from several microstimulation experiments. The experiments were carried using the following protocols: in half of the trials, microstimulation of area 3b of S1 substituted for the mechanical, comparison stimulus frequency (Romo et al 1998). Artificial stimuli consisted of periodic bursts

FIG. 5. Psychophysical performance in frequency discrimination with natural, mechanical stimuli delivered to the fingertips and with artificial, electrical stimuli delivered directly to S1 neurons. Monkeys were first trained to compare two mechanical stimuli presented sequentially on the fingertips (Fig. 1). Then some of the mechanical stimuli were replaced by trains of electric current bursts microinjected into clusters of QA neurons in area 3b. Each burst consisted of two biphasic current pulses. Current bursts were delivered at the same comparison frequencies as natural stimuli. In half of the trials the monkeys compared two mechanical vibrations delivered on the skin; in the other half one or both stimuli were replaced by microstimulation. The two trial types were interleaved, and frequencies always changed from trial to trial. The diagrams on the left show four protocols used. The curves on the right show the animals' performance in the situations illustrated on the left. Filled and open circles indicate mechanical and electrical stimuli, respectively; continuous lines are fits to the data points. (a) All stimuli were periodic; the comparison stimulus could be either mechanical or electrical. (b) The base stimulus was periodic and the comparison aperiodic; the comparison could be mechanical or electrical. (c) All stimuli were periodic; the base stimulus could be mechanical or electrical. (d) All stimuli were periodic; in microstimulation trials both base and comparison stimuli were artificial. Vibrotactile stimuli were either sinusoids or trains of short mechanical pulses, each consisting of a single-cycle sinusoid lasting 20 ms. Monkeys' performance was practically the same with natural and artificial stimuli. (Modified from Romo et al 1998, 2000.)
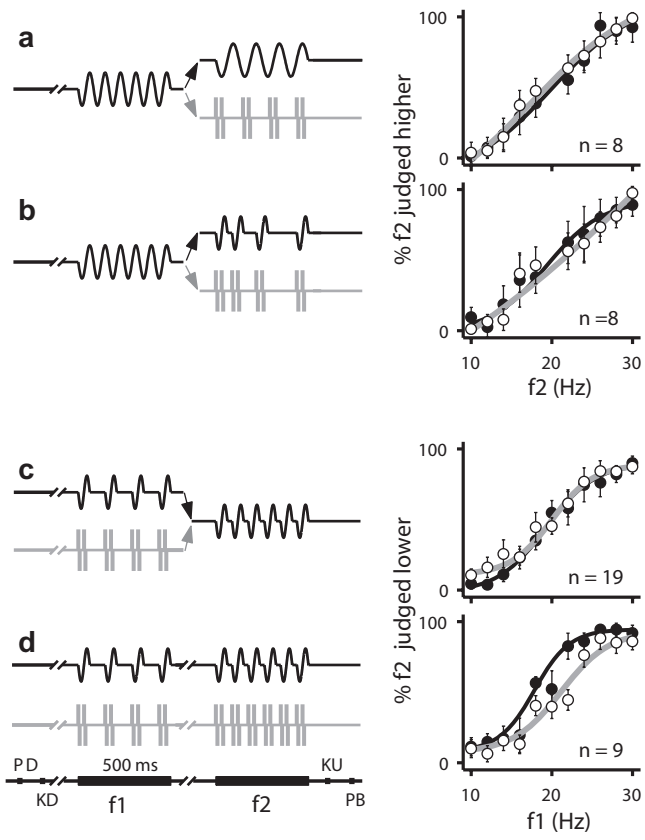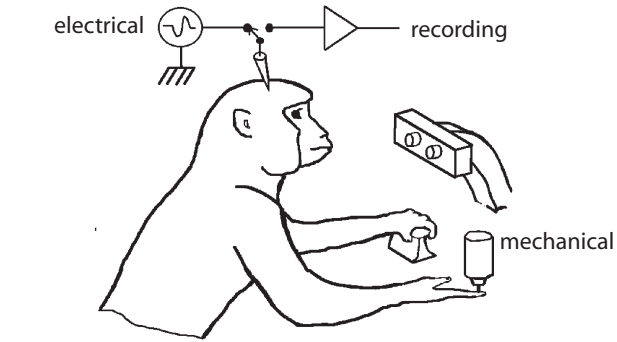
delivered at the comparison frequencies as the mechanical stimuli. Microstimulaion sites in area 3b were selected to have QA neurons with small receptive fields on the fingertips at the location of the mechanical stimulating probe. Remarkably, the monkeys could discriminate between the mechanical (f_1) and the electrical f_2 (comparison) stimuli with performance profiles indistinguishable from those obtained with natural periodic stimuli (Fig. 5a). Similar performance levels were observed with aperiodic stimuli (Fig. 5b). The most direct interpretation of these findings is that the electrical stimuli induced sensations that closely resembled those induced by the mechanical stimuli, and that periodicity does not matter for discrimination. This latter observation is consistent with the fact that neurons from areas central to S1 encode the stimuli in their firing rates and that their discrimination thresholds calculated from their firing rate match the psychometric thresholds (Hernández et al 2000, Salinas et al 2000).

Due to the task design, comparison of f_2 is made against the memory trace of f_1. We wondered whether in addition to using artificial stimuli during the comparison period of the task, monkeys could store and use a quantitative trace of an electrical stimulus delivered to clusters of QA neurons in S1 in place of the f_1 mechanical stimulus. We also wondered whether monkeys could perform the entire task on the basis of purely artificial stimuli (Romo et al 2000). This would demonstrate that activation of the QA circuit of S1 was sufficient to initiate the entire cognitive process involved in the task. In experiments in which the f_1 consisted of electrical microstimulation, the monkey's psychophysical performance was again indistinguishable from that quantified with only natural stimuli, indicating that the signals evoked by mechanical and artificial stimuli could be stored and recalled with same fidelity (Fig. 5c). Moreover, monkeys could perform the entire task, with little degradation in performance, on the basis of purely artificial stimuli (Fig. 5d).

As for substituting the comparison stimulus with electrical patterns, monkeys could not reach the usual level of performance when clusters of slowly adapting (SA) neurons were microstimulated (Romo et al 2000). Nor they could discriminate when microstimulation patterns were made at the border between QA and SA clusters. These control experiments tell us about the specificity of the QA circuit in this task (Romo et al 2000). This indicates that microstimulation elicits quantitative discriminable percepts, and shows that activation of the QA circuit of S1 is sufficient to initiate the entire subsequent neural process associated with vibrotactile discrimination. Relevant to interpreting the S1 microstimulation results, previous studies have shown that activity in a single cutaneous afferent fibre could produce localized somatic sensations (Johansson & Vallbo 1979), and frequency microstimulation of RA afferents linked to Meissner's corpuscles produced the vibrotactile sensation (Ochoa & Torebjork 1983). These observations strongly support the notion that the activity initiated in specific mechanoreceptors is read out by S1; this reading is then widely distributed to those anatomical structures that are linked

to S1. The whole sequence of events associated with this sensory discrimination task must depend on this distributed neural signal as already discussed in previous sections.

General discussion

The evidence reviewed here suggests that the comparison between stored and ongoing sensory information takes place in a distributed fashion. It also suggests that there is a continuum between sensory-and motor-related activity. For example, f_1 is encoded in multiple cortical areas. Such encoding seems to proceed in a serial fashion from S1 to S2, PFC, VPC and then to MPC. Although the strength of this signal varies across these areas, all of them except for S1 store f_1 at different times during the working memory component of the task. This is consistent with the proposal that there is a large cortical network that dynamically stores sensory information during working memory (Fuster 1997). During the comparison period, f_2 is processed similarly by the same cortical areas. The comparison between the stored sensory information of f_1 and the current sensory input of f_2 is observed in S2, PFC, VPC and MPC, again with various strengths across the cortical areas. This comparison signal evolves into a signal that is consistent with the motor choice; this is again stronger in some areas than in others, but is widespread nonetheless. The resulting motor signal is also observed in M1, but it does not seem to participate in the sensory, memory, and comparison components of the task.

   This comparative analysis shows that in the vibrotactile task, S1 is predominantly sensory and M1 is predominantly motor, but otherwise there is broad overlap in response characteristics across all other cortical areas studied. The difference between S2, PFC, PVC and MPC might best be characterized as shifts in the distributions of response types (Figs. 3, 4). For example, compare PFC, VPC and MPC: their response latencies were significantly different, with the f_1 and f_2 signals beginning slightly earlier in PFC and PVC than MPC (Romo et al 2004). The percentages of neurons that encoded each component of the discrimination task were also different. These findings suggest that the premotor areas may coordinate the sensory, memory and decision components of the task but that these processes are first coordinated in PFC and VPC. This result, however, should be interpreted cautiously, since recordings were made in different animals and the same population from each cortical area may vary from animal to animal.

   An interesting finding worth detailed discussion is the existence of neural populations with opposite responses—or, more precisely—of populations with opposite-sign tuning curves (positive and negative slopes). One of the simplest ways in which neurons could encode the frequency of vibratory stimuli is by means of a tuning curve in which particular firing rate values encoded particular stimulus frequencies, determined by any arbitrary function (Romo et al 2003). Then, if all

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neurons of a given area had similar responses, pooling of individual responses could provide an accurate estimate of the stimulus frequency (the fidelity of this estimate would be determined by the correlation values among neurons; Shadlen & Newsome 1998). Instead of this simple coding scheme, the results showed that, in all areas central to S1, there is not a single, but a dual stimulus encoding. Given that the slopes are of opposite signs (antagonistic responses), pooling the activity of these two groups of neurons would not give any useful information about the stimulus frequency. Therefore, well-structured cortical circuits are necessary to keep the information of each separated population. As we have seen, this dual encoding is preserved along the processing levels, from S2, PFC, PVC and MPC. What is the role of this dual representation?

It has been shown that responses of individual S2 neurons provide less information about the stimulus frequency than individual responses of S1 neurons (Salinas et al 2000, Romo et al 2003). Unlike S1, where the information provided by individual neurons is enough to explain the monkeys' discrimination thresholds, neurometric curves obtained from individual responses of S2 neurons are well below the discrimination thresholds of monkeys (Romo et al 2003). Is sensory information degraded as it flows from S1 to S2? At first sight, this may seem to be the case. However, combining the responses of neurons with opposite slopes could compensate for the loss of information. Indeed, we have shown that it is possible to recover the information apparently lost between S1 and S2 by means of a subtraction operation between pairs of neurons with opposite tuning curves (Romo et al 2003). This operation, which can be thought of as a contrast enhancement mechanism, is particularly useful when neurons show positive correlation coefficients: subtracting the activity of two positively correlated neurons cancels correlated random modulations. Thus, the existence of neuronal populations with opposite signs constitutes a mechanism for representing sensory information along the successive processing stages of cortex, even though significant levels of positive correlation exist among the activity of the neurons. Importantly, this encoding scheme has also been found in the cortices of monkeys that require behavioural decisions based on sensory evaluation (Sinclair & Burton 1991, Freedman et al 2001).

Concluding remarks

The highly simplified sensory discrimination task used here requires perceiving a stimulus, storing it in working memory, combining the stored trace with the current sensory stimulus and producing a decision which is communicated to the motor apparatus. The entire sequence of the task is reflected in the activity of neuronal populations from several cortical areas of the parietal and frontal lobes. Our results indicate that neurons from areas central to S1 do not simply wait for a signal encoding decision, but participate at every step of its generation by combining working

memory and sensory inputs. This process is carried out by two complementary neuronal responses. This dual representation is found in all areas central to S1 examined in this task, and might serve to compute optimally the entire perceptual process of the task. This coding scheme has been also found in some cortices of monkeys performing tasks that require behavioural decisions based on a comparison operation. An important problem posed by these findings is whether each neuronal correlate found in each cortical area actually has an impact in the perceptual task. Perhaps, microstimulation experiments of the type carried out in S1 are necessary to prove whether this is so.

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DISCUSSION

Derdikman: According to some theories, when you get to M1 you develop a population vector. Can you think of a population vector already existing in S2, for instance?

Romo: It may be possible, but our push buttons are 2 cm apart. The population vectors described in M1 do not apply to our experimental conditions. Our differential responses are related to the discrimination outcomes, rather than to motor outputs. In fact, the differential responses observed during the discrimination task are no longer present when animals perform push-button presses in a variant of the discrimination task that does not require somatosensory discrimination.

Brecht: This negative tuning is something new to the secondary cortex. It isn't seen in primary cortex. Is it seen in naïve animals?

Romo: Yes, S2 transforms the S1 positive tuning into a dual representation reflected in two separate neuronal populations: one that shows positive tuning and another that shows negative tuning. These two neuronal populations are necessary for the discrimination process (Romo et al 2003). They compute a subtraction operation that enhances sensory information which matches the animal's discrimination threshold. This neural operation, I believe, is forged during the learning process. Of course, this is difficult to prove because we have never tried it in naïve animals. This could be explored with chronic recording techniques, a problem which is currently addressed in our laboratory.

Brecht: In an anaesthetized monkey, do you see negative tuning in S2?

Romo: Most of the tuning responses beyond S1 are manipulated by the behavioural context. When monkeys do push-motor presses during a visual instruction task and the same stimuli are delivered to the fingertips, the tuning responses of S2 neurons are dramatically affected. I would assume that in anaesthesia these tuning responses are lost. However, we have not tested this.

Dehaene: Did you try to manipulate the duration of the stimuli to see whether it is frequency, number or duration that is important?

Romo: Yes and the results suggest that it is the firing rate. By analysing the responses of single neurons recorded in primary somatosensory cortex while trained monkeys discriminated between two consecutive vibrotactile stimuli, we tested five possible candidate codes. Monkeys could discriminate the difference in the frequency of two stimuli by measuring: (1) the time intervals between spikes; (2) average spiking rate during each stimulus; (3) absolute number of spikes elicited by each stimulus; (4) average rate of production of bursts of spikes; or (5) absolute number of spike bursts elicited by each stimulus. We found that each of these codes carries sufficient information about stimulus frequency to account for psychophysical performance. However, only a spike count code where spikes are integrated over a time window that has most of its mass in the first 250 ms of each stimulus period, covaried with behaviour on a trial-by-trial basis; was consistent with psychophysical biases induced by manipulation of stimulus duration; and produced neurometric discrimination thresholds similar to behavioural psychophysical thresholds. We also know that firing rate is the only code for sensory discrimination in

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areas central to S1. Therefore, firing rate seems to be the neural code to solving the vibrotactile discrimination.

The sensory component has an early latency and that proceeds from in a serial fashion, but the comparison component is longer relative to the pure sensory processing. The comparison component, by definition, is the result of the combination of the current sensory input with the working memory component. The latency of this operation is in the order of 180 ms, and that varies as a function of discrimination process.

Harris: You showed the functions for the S2 neurons, relating the frequency of the vibration to the firing rate response. It was basically linear in each case, whether they were ascending or descending. The comparison process seemed to be characterized by a subtraction.

Romo: That is the operation we propose. We have published these results in *Neuron* (Hernández et al 2002, Romo et al 2004) and in *Nature Neuroscience* (Romo et al 2002).

Harris: Would that imply that the discrimination ability should be invariant with respect to base frequency, comparing 12 against 14 would be the same as 22 against 24? In other words, you won't get a Weber fraction, but a flat function.

Romo: It's possible. I have done that comparison as a function of the difference of the frequency of the second and the first, and found two different functions. One that shows the difference between the first and second, then they become very categorical. And another function that simple tells you the categorical response. The first function must be more related to the sensory decision component, whereas the second one is more likely related to the decision motor report.

Harris: If you varied the difficulty, would this have an effect?

Romo: If we compare 20 Hz versus 20 Hz, it is very difficult to see this comparison signal in S2. But, if the difference between the two stimuli is 4 Hz, neurons reflect this. The sensory decision signal grows as a function of the difference between the two stimuli.

Harris: If you had 20 versus 22 and 20 versus 28, would there be much difference.

Romo: As I already told, some neurons reflect the comparison discrimination process.

Porro: Do positively or negatively tuned cells tend to cluster in different columns?

Romo: We have some ideas in S2. We have already seen those two populations, which seem to be arranged in clusters of neurons and tried to manipulate them through microstimulation as we did for S1. The result we found was quite strange. In microstimulation of a cluster of neurons that showed negative tuning, no matter what stimulus is used the animal has a tendency to report that the second stimulus was lower than the first. In our work, when a monkey makes three or four errors in a row, they change their strategy. Now, during microstimulation in a cluster of

neurons with positive slope, we observed exactly the opposite. This is very strange, and I haven't been able to pursue this problem. The recording and microstimulation observations suggest that S2 is organized in clusters of neurons that may correspond to what are called 'columns' in S1. We should focus on this problem.

Porro: There are suggestions from human studies that some kinds of somatosensory discrimination tasks involve the posterior parietal cortex. Have you looked there?

Romo: I simply showed the results obtained of few cortical areas. We have recorded from a lot more. In brief, information from S1 is widely distributed in the parietal lobe. Most of the neurons from parietal somatosensory fields have the tendency to encode the basal stimulus with the type of responses we observed in S2. Of course, there are many neurons that simply fire to the stimulus and do not encode a stimulus feature. We don't know the contribution of these neurons to the frequency discrimination task.

Porro: Are neurons in the posterior parietal cortex less precisely tuned?

Romo: No. There are neurons from area 7B, 5 or 2 that can be as good as the neurons from S2. However, neurons with no tuning functions can be also observed in S1.

Romo: Yes, we need more frequencies, and the study is now limited to the flutter range which is from 5–50 Hz. We are trying the flutter-vibration range and see whether that linearity is preserved or not.

Rizzolatti: At least from this plot, the behaviour of ventral premotor neurons seems to be very similar to S2 neurons.

Romo: The plot shows the relevant response during the base, working memory and comparison periods. The difference between S2 and ventral premotor cortex is the working memory component. S2 neurons are tuned to base stimulus, then the response is prolonged to the delay period between the two stimuli for about 500 ms. In the ventral premotor cortex you can have tuning during the base stimulus that is preserved along the whole delay period. There are some neurons that do not respond to the base stimulus, but they start to fire just before the second stimulus. It may be an expectation signal according to psychologists, but when we do the analysis we observe a gradient response linearly related to the base stimulus. Therefore, there is substantial difference in the response patterns of neurons from S2 and premotor cortices and of course with primary motor cortex.

Schall: The latency of the comparison response struck me. Are any of the cells responding early in an undifferential manner? Or are they differential from the beginning of the response?

Romo: The beginning of the differential response is about 200 ms. This is more or less the type of response you have measured in the frontal eye field. Apparently, there is something common which might be independent of the sensory modality. If I were there in the VPC with a visual task I probably could see this type of

response. I want to believe that these neurons might response to many sensory modalities, but it is very difficult to test the animals with more than one task.

Schall: If it is delayed according to the difficulty of the discrimination, then the latency of the response should be delayed when the stimuli are closer together.

Romo: We can measure this in the comparison process, but during the comparison process we have sensory responses with very short latencies. The differential responses grow as a function of time during the comparison period.

Logothetis: Were these recordings done from different areas done simultaneously?

Romo: We are now trying this by recording simultaneously neurons in S2 and frontal cortex.

Logothetis: If you have simultaneous recordings it will be interesting to apply some kind of modelling, to see whether from two or three neurons tuned to two different frequencies you have a good way to predict what is happening with cells at the single spike level.

Romo: We are analysing the data collected with the two implanted apparatuses.

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