Neural Correlates of Novel Object and Novel Location Recognition Behavior in the Mouse

Anterior Cingulate Cortex.

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Abstract

The anterior cingulate cortex (ACC) is a component of the limbic system implicated in a wide variety of functions spanning motor and sensory information processing, memory, attention, novelty detection, and comparisons of expectation versus outcome. It remains unclear how much of this functional diversity stems from differences in methodology or interpretation versus truly reflecting the range of processes in which the ACC is involved. In the present study, ACC neuronal activity was examined in freely behaving mice (C57BL6/J) under conditions allowing investigation of many of the above functions in conditions free from externally applied rules: tests of novel object and novel location recognition memory. Behavioral and neuronal activity was recorded first in the open field, during the initial exposure and subsequent familiarization to two identical objects, and finally during the recognition memory tests. No discernible stable firing correlates of ACC neurons were found in the open field, but the addition of objects led to lasting changes in the firing patterns of many ACC neurons around one or both of the object locations. During the novel location test, some neurons followed the familiar object to its new location, others fired exclusively where the object had been, and yet others fired to both current and former object locations. Many of these same features were observed during tests of object recognition memory. However, the magnitude of the neuronal preference for the novel or the familiar object was markedly greater than that observed during either the tests of location recognition or novel object preferences in animals that did not exhibit the expected behavior. The present study reveals, for the first time, single neuron correlates of object and location recognition behaviors in the rodent ACC, and suggests that neurons of the ACC provide a distributed representation of all of the salient features of a task.
Introduction

The cingulate cortex is one of the major parts of Broca’s “limbic lobe”, a set of evolutionarily-conserved interconnected medial forebrain structures found in all mammals. The cingulate cortex is more difficult than most major limbic areas to succinctly ascribe a function to, compared with, for example, the association of the hippocampal formation with memory or the amygdala with emotion. This is not because of any lack of functional correlates, but is instead due to the sheer breadth of functional roles ascribed to it. The anterior portion of the cingulate cortex (ACC) alone has been associated with a range of functions from sensorimotor processing (e.g., Carmichael and Price 1995; Isomura and Takada 2004) and pain (e.g., Wang et al. 2003) to more complex cognitive functions such as novelty detection (e.g., Downar et al. 2002; Downar et al. 2000; Kabbaj and Akil 2001), attention (e.g., Fan et al. 2005) and determination of salience (e.g., Downar et al. 2003; Weible et al. 2003), action/outcome valuations (e.g., Rudebeck et al. 2006b; Rushworth et al. 2004), social interest and the utilization of social information (e.g., Rudebeck et al. 2006a; Rudebeck et al. 2007), memory (e.g., Frankland et al. 2004; Frankland et al. 2006; Teixeira et al. 2006), comparisons of expectation versus outcome (e.g., Johnston et al. 2007), and representation of salient features of the task (e.g., Fujisawa et al. 2008; Lapish et al. 2008). While a number of studies have examined ACC electrophysiological correlates of cognition and behavior, certain factors can complicate interpretation of the data. In many studies, recordings were made in highly trained animals, making it difficult to differentiate between innate and learned neuronal responses. In others, interpretation of the data requires consideration of the impact of externally applied rules. These problems are ameliorated when employing tests of recognition memory, where one can realistically record neuronal responses throughout all aspects of the task.

Novel object and novel location recognition tests (Ennaceur and Delacour 1988) are simple behavioral assays of memory that rely primarily on a rodent’s innate exploratory behaviors in the absence of externally applied rules or reinforcement. The animal is familiarized to particular objects in particular locations and then presented with either a novel object in a familiar location or a familiar object in a novel location, respectively. The preference for novelty is revealed by the tendency of the animal to spend more time exploring the novel, versus the familiar, stimulus. For this reason, object and location recognition tests are particularly well suited to the approach applied to the study of “place cells” in the rodent hippocampal formation: relating neuronal activity to an animal’s location in space. While the majority of studies with this task involve rodents (Akirav and Maroun 2006; Ennaceur et al. 1997; Hammond et al. 2004; Yee 2000), there are clear cognate versions in other species, including humans (Pihlajamaki et
al. 2005; Pihlajamaki et al. 2004) and primates (Murray and Mishkin 1998; Parkinson et al. 1988), facilitating the comparison of data across species. Together, these two tasks provide straightforward, ethologically valid, tests of spatial and non-spatial memory.

The present study was undertaken to test the hypothesis mentioned above (Fujisawa et al. 2008; Lapish et al. 2008) that the ACC represents salient features of the task. To examine how the individual elements of the task are represented prior to the tests themselves, neuronal data were collected during open field exploration, initial exposure to objects, and multiple re-exposure sessions intended to completely familiarize the mice to the objects and their locations. In this way, distinctions between innate and learned neuronal responses may be drawn, as well as the manner in which many of the other hypotheses mentioned above may be reflected in the neural representation. For example, if ACC neurons fire to the object locations, they could be responding to the object identities themselves, much as the object cells of inferotemporal cortex (Chelazzi et al. 1998). If ACC neurons are responsive to novelty, initial exposure to objects will result in a marked reorganization of neural activity compared to that observed during exploration of the open field. If such responses are novelty-specific, they would subsequently disappear as the objects become familiar. Alternatively, if the ACC is involved in determining salience and mediating attention, object exploration and neuronal activation would continue to correlate after novelty has been lost. The tests for object and location recognition followed the re-exposure sessions. If ACC involvement in novelty detection is generalized, neuronal correlates of the recognition behavior should be evident during both memory tests. If, instead, the ACC is involved specifically in object or location recognition, a change specific to one or the other test should be evident. Additional information regarding ACC function will also likely be gained from these recordings. For instance, a mnemonic representation of the environment would involve conjunctions of objects and locations. Rodents exhibit persistent exploratory behaviors in previously salient locations (Save et al. 1992a). Based on evidence for ACC involvement in memory and comparisons of expectation versus outcome, persistent exploratory behavior of the former object location during the test of location recognition, if observed, should be reflected in the ACC. In each of these cases, changes should be detectable at the single neuron (e.g., an increase in firing rate) and/or the population (e.g., an increase in the number of responsive neurons) level.

The data presented here clearly demonstrate that, while ACC neuronal activity is not intrinsically spatial, a robust correlation exists between ACC neuronal activity and behavior associated with object exploration. Specifically, the activity of individual ACC neurons persistently reflects the locations of one or both objects, where
objects had previously been, and the behavior associated with object recognition memory. The correlates observed reflect many of the processes associated with the ACC, and lend support to the recently proposed hypothesis that, at the network level, the ACC reflects salient features of the task (Fujisawa et al. 2008; Lapish et al. 2008).

Methods

30 male C57Bl6/J mice were implanted with an adjustable-depth 4-tetrode array for the purpose of recording the activity in vivo of ACC neurons during spontaneous exploration of a cylindrical arena and intra-arena objects. All procedures described were performed in accordance with guidelines approved by University of Oregon’s Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

Surgical Procedures

All surgeries were performed using aseptic techniques. Ketamine (100mg/Kg) was administered as a pre-anesthetic. Dexamethasone (0.1mg/Kg) and atropine (0.03mg/Kg) were administered pre-surgically to ameliorate possible inflammation and respiratory irregularities, respectively. Surgical anesthesia was maintained with isoflurane (1.25-2.0%, adjusted as necessary for appropriate depth of anesthesia). Eyes were kept moist with a thin layer of antibacterial ophthalmic ointment. Mice were positioned in a stereotaxic frame. The skull was then exposed, and lambda and bregma zeroed in the vertical plane. One hole was drilled in the left hemisphere, centered over the caudal anterior cingulate cortex (see below) (AP: +0.6mm, ML: 0.5mm, relative to bregma), through which tetrodes from a custom built adjustable depth recording array were passed (DV: -1.0mm, relative to dura). Two additional holes were drilled for the insertion of stainless steel screws (00-90 x 1/8”) to ground and anchor the recording array. Grip Cement (Dentsply, Milford DE) was used to secure the array to the skull. Vaseline was applied to isolate the individual tetrodes from the cement, preserving the ability to adjust tetrode depth. Mice were administered buprenorphine (0.06 mg/Kg, s.c.) post-operatively for analgesia to minimize discomfort. All mice were individually housed following surgery due to the delicacy of the implanted recording arrays.

Behavioral Training

All mice were allowed to recover from surgery for 7 days before handling. Screening for units was then performed in the home cage. For the purposes of monitoring/recording behavior and ACC neuronal activity, a tethered HS-16 operational amplifier (Neuralynx, Bozeman, MT) was plugged into the EIB-16 electrode interface.
board (Neuralynx, Bozeman, MT) of the tetrode recording array. If the neuronal activity observed was of insufficient quality, the array was moved down ~45 µm, and the animal was returned to the colony room. When activity of sufficient quality, subjectively defined as the separation of individual waveform clusters (see Data Analysis, below), was observed, data collection in a cylindrical arena would begin. All subsequent behavioral/neuronal recording sessions were 10 minutes in duration. Between all sessions, the mouse was transferred from the cylinder to the home cage, placed in an opaque transport box, and removed from the recording room. Brown butcher block paper beneath the cylinder was discarded and the cement floor beneath wiped down with 90% EtOH. The cylinder was likewise wiped down with 90% EtOH then replaced in the original position and orientation upon a fresh sheet of paper. Objects, when present, were also wiped down with 90% EtOH between sessions. Objects were fixed firmly to the paper with a dual-sided strip of carpet tape.

*Initial Exposure*

Mice were placed in a cylindrical arena, set upon a sheet of paper. The cylinder was 60cm in diameter and 45cm in height, painted black with white geometric shapes stenciled on the inner surface, and was surrounded by an un-cued ceiling-height black curtain. Four equally spaced lights directly above the cylinder provided illumination. All entries into the cylinder were from the same position (South). All positional and neuronal data were recorded for each session as described below in Methods: Single Neuron Recording. The first two Open Field sessions involved exploration of an empty cylinder. This was followed by Initial Exposure to two identical objects.

*Object/Location Familiarization*

Following Initial Exposure, four additional 10-minute daily recording sessions were carried out. These four Re-Exposure sessions with the same two identical objects in the same locations relative to starting position were intended to fully habituate the animals to the objects and the environment. On a sixth day of recording, six consecutive 10-minute sessions were performed. The six sessions were identical to each other and all preceding object exploration sessions. Sessions were separated by approximately 6 minutes (the time required to reset between sessions). Recordings of behavioral and neuronal activity during these six Control Block sessions served to identify changes in activity across multiple sessions in the absence of environmental changes, in anticipation of blocks of consecutive sessions during which objects and their locations were manipulated (Test Block sessions), as well as to familiarize animals with repeated handling.
Object/Location Recognition Tests

On the final day of recording, mice were separated into two groups and tested for novel object and novel location recognition behavior. The sequence of these tests was counter-balanced such that mice were tested first for either object recognition or location recognition. Each sequence involved a six session Test Block, with sessions 1 & 2 identical to all preceding familiarization sessions. During session 3, the first test of novelty, either object or location, was performed (session 4 was identical to session 3). During session 5, the second test of novelty was performed (session 6 was identical to session 5). Figure 1 illustrates the sequence of all behavioral/neuronal recording sessions from the Open Field through the Test Block sessions. Scoring of object recognition behavior was done entirely from the positional signal during recordings. While this method may not capture the actual orienting behavior as accurately as a trained behavioral scorer does, it would not have been possible to synchronize a scorer’s output with the millisecond timescale required for the electrophysiology. The net effect is to include more noise in the behavior, making it more difficult to obtain significant data, but no difficulties were encountered in obtaining significant neuronal correlates with a purely positional signal.

Single Neuron Recording

Manufacture of the 4-tetrode recording array was adapted from methods described by Gray and colleagues (1995). Briefly, four lengths of 18µ diameter 10% Iridium/Platinum wire (California Fine Wire, Grover Beach, CA) were spun together and heated to fuse the polyamide-coating at one end. The coating on the free ends of each wire was removed, and each uncoated wire segment was inserted into a channel of an EIB-16 electrode interface board (Neuralynx, Bozeman, MT) and fixed into place with a gold-coated pin. Each EIB-16 loaded with four tetrodes was fixed to a Teflon stage mounted on three drive screws. The drive screws (0-80 x 3/8”) provided adjustability of depth of the array as a single unit, and served as the structural support for the array affixed to the skull.

Neuronal data were acquired using the Cheetah-16 system (Neuralynx, Bozeman, MT). Neuronal signals were buffered through the HS-16, passed via a 2-meter tether to the “ceiling” of the recording chamber. In an adjacent room the signal was amplified 10,000x and captured using Neuralynx data acquisition software. Thresholds were set such that only waveforms of a specified minimum voltage were stored. A digital camera fixed to the “ceiling” of the recording chamber linked to the Cheetah-16 system recorded the position of the animal during the course of each session by tracking two LEDs fixed to the HS-16. The behavioral sequence involved extensive familiarization with the objects in their spatial context. While an attempt was made to record the same ACC neurons
longitudinally throughout all parts of the task, spanning the Initial Exposure through the Test Blocks with the same units proved largely unsuccessful. If units were lost during either a single session (e.g., Re-Exposure) or a session block, the tetrode array was moved down following completion of all recordings for that day. Following days in which the tetrodes were moved, animals were rescreened in the home cage prior to being placed in the cylinder, and the tetrode moved downward again if acceptable activity was not observed. While this process did in some cases add to the total number of days required for an experiment to be completed, the total number of daily recording sessions did not vary, and all recordings were typically performed within a 2 week time frame.

Data Analysis

Rate maps of all behavioral and neuronal data were generated using the custom software package Session_Analysis (Agnihotri et al. 2004). Data corresponding to the 60 cm diameter cylinder were parsed into a 2D matrix 26 pixels in diameter for a total floor area of ~531 pixels. Areas 5 pixels on a side corresponding to each of the three object locations used were determined for each block of sessions. The average footprint of the objects used in the present study was 9.2 square pixels, and all objects fit in their entirety within the 5 x 5 pixel region. The average maximum object height was 3.8 cm. Individual objects are described in detail in Table 1. Because in some cases not all pixels within the object locations (e.g. those on the surface of the object) were explored, comparisons between object locations were performed using unpaired t-tests. In instances where coverage was such that no object-associated pixels were visited (e.g., cases of extreme neophobia in response to the objects, or extreme inactivity, seen more often during Initial Exposure to the objects), sessions from the respective block of sessions were excluded from subsequent analysis. All statistical analyses of behavioral and neuronal activity were performed with unsmoothed data. For illustrative purposes, all behavioral occupancy and neuronal rate maps were smoothed using a 3 x 3 box-car kernel. Behavioral analyses during recognition memory tests (Test Block sessions 3 & 5) involved unpaired t-test comparisons of the mean time spent exploring the novel and familiar object/locations, comparable to the method previously employed by Skov-Rackette and Shettleworth (2005).

The activity of individual neurons was isolated offline using the MATLAB (The MathWorks, Inc)-based spike sorting software MClust (A.D. Redish, University of Minnesota). Two dimensional plots of pairs of waveform measures were used to identify clusters corresponding to waveforms of individual neurons. The axes of cluster space used were spike height, valley, and energy. MClust provides the flexibility to apply cluster boundaries across multiple recording sessions. Neurons were judged to be the same from one session to the next if the same cluster
boundaries could be applied across consecutive sessions without compromising the isolation of the cluster. All analyses described in the present study for Open Field/Initial Exposure, day pairs during Re-Exposure, and Control Block and Test Block sessions, include only those data for which the same cluster boundaries could be applied (see Supplementary Materials Figure S1 for how cluster separation and stability were judged).

Unpaired t-tests were performed to compare firing rates between object locations and the background, as well as between pairs of object/locations. Twenty-five pseudorandomly selected pixels not associated with the three object locations represented the background firing rate. Locations 1 & 2 contained the two familiar objects (A₁ and A₂, respectively, in Figures 2, 5-8), with Location 2 becoming the position of the novel object (B₂ in Figures 2, 6-8). Location 3 was the novel location (A₃ in Figures 2, 6-8). The comparisons of object/location-associated firing rates to background were performed to identify increases and decreases in activity of ACC neurons across Initial Exposure, Re-Exposure, Control Block and Test Block sessions, as well as to identify those neurons exhibiting sustained responses across 2 or more sessions. The comparison between pairs of object locations was performed separately to identify those cells exhibiting significantly different magnitude responses between pairs of object/locations. Cells exhibiting this difference were said to have a “preference” for one object/location over the other. Z-scores of those differences were then calculated for group comparisons. \( \chi^2 \) tests were used to compare proportions of neurons exhibiting different preferences (e.g., between the novel and familiar objects), with the familiar condition as the expected value and the novel condition as the observed value.

Two additional analyses were performed on data collected during the two Open Field sessions on the first day. First, analyses were performed to determine whether ACC neurons exhibited “place fields” analogous to those first observed by O’Keefe and Dostrovsky (1971). Measures included coherence, field size, and information content. Coherence was calculated as the z-transform of the correlation coefficient between the cell’s firing rate in a given pixel and the mean in the eight closest pixels; field size was calculated from areas with at least four contiguous pixels of firing; and information content was calculated as \( \sum P_i \log_2 \left( \frac{R_i}{R} \right) \), where \( i \) is the bin number, \( P_i \) is the probability for occupancy of bin \( i \), \( R_i \) is the mean firing rate for bin \( i \), and \( R \) is the overall mean firing rate (Markus et al. 1994). To test for stability across the two open field exploration sessions, similarity scores were calculated by converting rate maps into two columns of data points and calculating the correlation coefficient (Pearson’s linear correlation). Pixels of incongruity between the two columns, resulting from non-visited pixels in one or the other session, were eliminated from the similarity score calculation. Second, the rate maps were divided into three regions:
a central zone 6 pixels in radius (~113 pixels), an intermediate ring extending from 7 to 10 pixels from the center (~201 pixels), and an outer ring 3 pixels in width (~217 pixels). Unpaired t-tests were performed to compare firing rates between the three regions. These analyses were performed initially on all data collected during the two 10 minute Open Field sessions. Data from cells exhibiting a similar response profile across the two sessions were then filtered at three different exploratory speeds based on the work of Granon and colleagues (2003): slow (<6.8 cm/sec), medium (6.8 cm/sec – 14.4 cm/sec), and fast (>14.4 cm/sec). Analyses of mean firing rate between regions and across different running speeds were performed with paired t-tests.

Results

The purpose of the present study was to examine how the activity of individual ACC neurons correlated with the behavior of mice exploring both familiar and novel objects. This was accomplished by recording positional data of the mice as they explored first an empty arena, and then the same arena with objects and comparing neuronal activity in the object locations to background. The sequence of recording sessions is summarized in Figure 1. Robust correlations between ACC neuron activity and exploratory behavior were observed at multiple phases of the study. Of particular interest were patterns of ACC neuron activation observed during expression of novel object recognition behavior but not apparent during tests of novel location recognition.

Behavior

Following identification of neurons with activity of sufficient quality, animals were allowed to explore the empty arena twice (Open Field sessions), following which two identical objects were placed in the arena for Initial Exposure. Mice were then re-exposed to the objects across subsequent days in order to 1) test for behavioral and neuronal habituation and 2) to ensure complete familiarity to the objects in their locations. Despite extensive familiarization, mice never completely habituated to the objects behaviorally, exhibiting greater dwell times in the object locations (e.g. session 1 of the subsequent Test Block; df = 16, t = 5.157, p < .0001). However, compared to Initial Exposure, exploratory behavior did attenuate, with mean object-specific dwell time per pixel decreasing by almost half (df = 24, t = 4.037, p = .0004).

Following the Re-Exposure and Control Block sessions, the animals were tested for both novel object and novel location recognition behavior. These tests provide a way to quantify an innate preference for novelty exhibited across species, reflected by greater time spent exploring a stimulus with novel characteristics (identity or location),
and require a comparison of incoming sensory input with expectation based on an internal representation of the environment. The tests were performed in series, so the testing sequence was pseudorandomly varied to control for order effects. Therefore, two behavioral groups were defined by whether the novel object task preceded or followed the novel location task. The testing sequences are illustrated in Figures 2A & 2E. While the mice generally exhibited the expected behavior, the sequence in which these tests were administered did impact upon performance. No behavioral preference for one or the other familiar object was observed during session 1 for either group (Figs. 2B & 2F). When object recognition was tested first, both tests elicited the expected preference for novelty \((df = 9, t = 3.475, p = .0052)\) and \((df = 9, t = 2.289, p = .0428)\), respectively, Figs 2C & 2D. In contrast, when location recognition was tested first, the preference for location novelty \((df = 6, t = 3.530, p = .0124; \text{Fig 2G})\), was not followed by the expected preference for object novelty (Fig 2H). At least part of the reason for this may be related to the animals’ tendency to continue exploring, during sessions 3 through 6, the region of the arena floor in which the familiar object had been \([F(1,12) = 5.219, p = .0413]\). The animals appeared to continue to react to the location shift even when the novel object was subsequently introduced, so the serial protocol in essence set up a competition between two kinds of novelty during the second test, rather than simply a comparison of a novel object to a familiar one, potentially explaining this order effect. These data indicate that, in general, the expected behaviors were observed. However, persistent investigation of previous object locations and the failure to exhibit object recognition behavior following the test for location recognition were unexpected. How these behavioral observations relate to the activity of individual ACC neurons is detailed below.

**ACC Neuronal Analyses**

*Histology*

Stable recordings histologically verified as reflecting the activity of ACC neurons were obtained from 19 of 30 mice during Open Field/Initial Exposure, the Control Block and/or the Test Block sessions. The histology showing recording sites in caudal anterior cingulate is illustrated in Figure 3. Only data from session blocks for which recording stability and arena coverage requirements (see: Data Analysis) were satisfied were included in the subsequent analyses. Behavioral and neuronal data from the remaining 11 mice were excluded from subsequent analyses.
In the Open Field, ACC units had a relatively low mean firing rate (3.96 Hz ± 0.57 SEM), firing more on average in some parts of the environment than others. As expected, the majority of neurons exhibited little spatial correlation in activity between the two Open Field sessions (i.e. they did not fire in the same places). Combined with measures of coherence, sparseness and information content, these data (Figure 4; see also Supplementary Materials Figure S2) indicate that mouse ACC neurons do not exhibit spatial correlates such as those found in other parts of the brain (e.g., “place” cells of the hippocampus). An effect of running speed during these sessions was also tested for (after object addition, running speed is conflated with object exploration), but no correlation was found between running speed and unit activity in the Open Field (\( r = 0.0493 \pm 0.0134 \), mean±/SEM). Though spatial correlates in the traditional sense were not observed, some (13%) cells did exhibit a discernible and reproducible firing pattern: a significantly greater firing rate in the center of the cylinder compared with the outer edges (Figure 5). These “bull’s-eye” cells responded stably across the two Open Field sessions, and exhibited a higher mean firing rate (8.12 Hz ± 1.66 SEM) compared with the remaining cells (3.47 Hz ± 0.65 SEM; \( df = 93, t = 2.855, p = .0056 \)). These cells were not obviously differentiable by type (Bartho et al. 2004), with no significant difference in spike widths (Henze et al. 2000) observed between bull’s-eye (513.6 µs ± 24.5 SEM) and the remaining (491.7 µs ± 17.8 SEM) cells. As expected, without filtering for run speed, the mean firing rate in the central zone (11.91 Hz ± 1.87 SEM) was found to be greater than that in the intermediate (10.07 Hz ± 1.76 SEM; \( df = 11, t = 5.245, p = .0003 \)) and outer rings (7.16 Hz ± 1.66 SEM; \( df = 11, t = 5.918, p = .0001 \)). This pattern was still apparent when the data were filtered for different rates of exploration (slow: <6.8 cm/sec; medium: 6 – 14 cm/sec; and fast: >14 cm/sec; data not shown). Similarly, the mean firing rates in the central zone, intermediate ring and outer ring did not differ significantly at each of the exploratory speeds examined (data not shown). Controlling for differences in firing rate between cells (dividing the firing rate in each region at each different exploratory speed by the overall firing rate of the cell for the 10 minutes session), relatively higher firing rates were found in the intermediate ring at the medium compared to the slow \( (t = 2.351, p = .0281) \) and fast \( (t = 2.159, p = .0420) \) exploratory speeds. These analyses indicate that ACC neurons do not exhibit spatial correlates in the classic sense. However, a small sub-population of ACC neurons was found to exhibit higher firing rate toward the center of the arena consistently across sessions.
Object-related Firing During Familiarization

Following the two Open Field sessions, two identical objects were placed onto specific locations of the arena floor (A₁ and A₂). Many neurons (24/95, see Table 2) clearly changed their firing rates in the object locations during Initial Exposure (e.g., Figure 5, Neuron 1). Since there was little tendency for these cells to fire specifically in these areas prior to the introduction of the objects, the firing appears to be associated with the objects rather than the locations, per se. Interestingly, some neurons responded to only to a single object (14/95) while others responded to both (10/95), and the strength of these responses did not differ significantly between the two groups. Since the objects were identical in all but location, this appears to reflect a conjunction of object identity and location in the activity of ACC neurons. In a subset of animals for which a second Initial Exposure session was run, 23% of neurons sustained their object related firing over the two sessions. The same basic pattern of neurons firing to one or both objects persisted throughout the next four daily Re-Exposure sessions with the initial pair of objects, indicating that the objects continued to effectively elicit neuronal responses.

On the sixth day mice underwent a block of 6 consecutive sessions with the same two identical objects in the same locations (Control Block; see Figure 1). These provided a parallel block of familiar condition control recordings for the novel object and novel location tests on the following day, as well as exposing mice to the stress associated with repeated handling over a relatively brief (~90 minutes for 6 sessions) period of time. Stable recordings of 134 neurons from 12 mice were collected across the 6-session Control Block. The majority of cells (Table 2) exhibited a significant change in firing rate at an object location in at least one of the six sessions. Of those, about half were responsive to only one object, while the other half were responsive to both objects. Responses were quite stable, with 48% of sustained responders exhibiting a similar response across four or more sessions. The amplitude of object responses as a population did not vary significantly over the six sessions.

The proportion of object-responsive neurons observed per session did not differ over the course of the experiment (see Table 2, column “Per Session”). However, a change in the magnitude of the response (ratio of object/location over background) was observed. For those mice from which both stable Initial Exposure and Test Block sessions data were collected, neurons exhibited a decrease in the magnitude of object-associated modulation ($df = 54, t = 2.103, p = .0401$). As noted above, there is an interesting behavioral correlate to this moderate habituation of response magnitude of ACC neurons upon repeated exposures to them: while animals still spent a greater amount of time exploring the objects relative to the rest of the arena during session 1 of the Test Block,
reflecting the intrinsic salience of the objects to the mice, they did so significantly less than during Initial Exposure, when the objects were truly novel (see Behavioral Analyses, above). These data indicate a decrease in the amplitude of object-associated responses between Initial Exposure and start of the Test Block sessions, but no change in the proportion of responsive neurons. Importantly, behavioral exploration of and neuronal responses to the objects remain highly significant compared to baseline.

Tests of Object and Location Recognition

For testing, the animals were divided into two groups to control for order effects. The analyses were performed on stably recorded data of 119 neurons from 10 mice tested first for object recognition memory, and of 104 neurons from 7 mice tested first for location recognition memory, summarized in Table 2. Neurons were observed exhibiting strong firing correlates to all relevant object locations during both tests. Remarkably, during the novel object task the neurons showed enhanced discrimination between the two objects only in those animals successfully expressing the appropriate novelty-seeking behavior. This heightened selectivity was not observed in those animals that failed to exhibit the novel object recognition behavior, or in animals exhibiting location recognition behavior. These results are discussed in greater detail below.

Object-Related Firing During the Novel Location Test

During the novel location test, an identical familiar object is displaced from its familiar location. Both sets of animals exhibited the preference for novelty, spending more time in the vicinity of the novel versus the familiar location. In the ACC, a number of neuronal firing correlates to object exploration were observed. Neurons were found with clear firing correlates to each of the three relevant locations, in different combinations and at different points in the task. Figure 6 illustrates three different response types observed on a single tetrode from a mouse tested first for location recognition memory. Although the data are analyzed entirely with regard to space, these are not simply location-specific firing properties. All three cells fire in different locations at different points in the sequence of sessions. Neuron 3 in Figure 6 fires specifically in the vicinity of the objects and faithfully follows the objects around the arena. Neuron 2 fires not only where the objects are, but also where an object had previously been. Neuron 1 never fires where an object actually is, and fires instead to where the trans-located object had previously been. These data demonstrate that moving a familiar object to a novel location (e.g., Figure 6, session 3; Figure 7, session 5) results in the reorganization of the spatial firing properties of ACC neurons such that some neurons fire where either one or both objects are, while others also or exclusively reflect where objects used to be.
During the test of novel object recognition, a familiar object is replaced with a novel one. The expected behavior was only observed when this test preceded that for location recognition. Those data will be discussed first. Figure 7 shows the rate maps of 2 units recorded from the same animal, with the behavior illustrated in the adjacent occupancy maps. In the familiar condition, Neuron 1 fires very clearly next to the two identical objects at roughly the same moderate rate. Neuron 2 fires in a relatively diffuse manner. However, when the novel object (B1) is introduced, Neuron 1 nearly doubles its firing rate at that location, firing only moderately near the familiar object (A1). During session 4, its firing rate is lower, though the response to both objects is still significant. Interestingly, Neuron 2 responds during the test not by firing more to the novel object but to the familiar object, and as with Neuron 1, this response is less pronounced during subsequent sessions. This pattern of activation was not observed when the test for object recognition followed that for location recognition (e.g., Figure 6, session 5), when mice did not exhibit the expected preference for object novelty. These data demonstrate that successful expression of object recognition behavior is associated with a specific pattern of neuronal activation. However, rather than simply mirroring the behavioral preference for the novel object (i.e., all responsive neurons firing more to the novel object), individual neurons exhibited a much greater preference for either the novel or the familiar object.

Neuronal Correlates of Recognition Behavior

As suggested above, one might expect a neuronal correlate of recognition behavior to simply reflect that behavior, such that the novel object or location would evoke a more robust neuronal response. This, however, did not appear to be the case. During tests of object or location recognition, the novel did not selectively evoke a more robust change than the familiar. Neither the strength of the responses to novel or familiar stimuli, nor the proportion of neurons exhibiting the different responses, differed between the tests of object or location recognition behavior. Analyses were also performed between sessions. During the test of location recognition, the responses observed in the novel Location 3 did not differ significantly from the response in Location 1 (from which the familiar object had been trans-located) during the session preceding the switch. In contrast, during expression of object recognition behavior, the firing rate of responsive neurons in Location 2 was moderately more ($df = 44, t = 2.366, p = .0240$) during the test compared with the response in the same location during the previous session. These data demonstrate that the activity of ACC neurons does not correlate directly with the behavioral preference for novel objects or novel
locations as measured during the test. However, the response to the position of the novel object is greater at test compared with the previous session when the behavioral preference is observed.

Interestingly, features distinguishing the object recognition performers from non-performers (i.e., greater neuronal preferences exhibited by individual neurons for either the novel or the familiar object) appear also to distinguish this group from animals exhibiting location recognition behavior. Only during successful expression of object recognition behavior did more neurons prefer the novel to the familiar (22 versus 14 neurons, $X^2 = 4.57$). The magnitude of the preference exhibited by these neurons for one or the other object increased quite markedly ($df = 58$, $t = 3.402$, $p = .0012$; see Figure 8A) compared to the preferences observed during session 1. Stated simply, individual neuronal preferences for one object or the other were significantly greater during object recognition behavior than during session 1, when both objects were familiar.

During object recognition behavior, 30% of neurons (36/119) exhibited a significant preference for either the novel or the familiar object (session 3). When the test for object recognition followed the test for location recognition, the condition in which object recognition behavior was absent, 23% of neurons (24/104) exhibited a preference for either the novel or familiar object during the test (session 5); this difference was not significant. However, the magnitude of the neuronal preference for one or the other object was much greater ($df = 58$, $t = 3.291$, $p = .0017$) when the behavioral preference for object novelty was observed as compared with when the behavior was absent. Both the novel and familiar objects elicited greater neuronal preference magnitudes ($p < .05$) during expression of object recognition behavior than either object when the behavior was absent. Interestingly, the neuronal preferences observed during object recognition behavior were also significantly greater than those observed during either test of location recognition. The object and location preference magnitudes for sessions 1, 3, and 5 are summarized in Figure 8. These data demonstrate that recognition of object novelty is accompanied by a pattern of neuronal activation not observed either when the behavior is absent, or during behavioral expression of location recognition. However, the consistent difference is in preferences exhibited by single neurons for either the novel or the familiar object.

Object Specificity and ACC Neuron Activity

Taken together, object/location-associated unit responses are remarkably abundant in the ACC. Fully 78% (173/223) of neurons exhibited a significant change in firing rate over baseline in one or more of the 3 pertinent object locations during at least one of the six Test Block sessions. The majority of these responses are indeed object-
associated, rather than attributable to chance. During the two Open Field sessions, when no objects were present, only 6% (5/77) of cells, excluding those identified as bull’s-eye cells, exhibited a significant location-associated change. Assuming an average of 2.5 cells per session, this suggests a maximum of 19% of “object-associated” responses may be attributable to chance. That these chance responses were significantly smaller in amplitude compared to baseline than object-associated responses observed during Initial Exposure to the objects ($df = 20, t = 2.745, p = .0118$) further emphasizes their distinction from actual object-associated activity. As described above, stability across sessions was robust during the Control Block. Similarly, during the Test Block, changes were sustained in location and sign for at least two consecutive sessions in 44% (98/223) of cells, with a majority (32%) of those increasing and a minority (12%) decreasing their firing rate around the object locations. The Test Block data are separated by group in Table 2. In contrast, only 1 neuron exhibited a change of the same sign in the same location during the two Open Field sessions. Arguing against a strict “object cells” interpretation of the data, however, is the observation that 49% (48/98) of cells exhibiting sustained responses during the Test Block session began doing so only after the object manipulations started in session 3. This includes nascent responses to familiar objects in both familiar and novel locations. There is additionally the tendency of a number of cells (26/223) to significantly modulate firing in the vacated position (Location 1) during and following the test of location recognition. These data, combined with evidence for the changes in firing patterns associated with behavioral demonstration of object recognition, suggests that the mouse ACC reflects multiple distinct aspects of these simple behavioral tasks, and in the aggregate help represent the animal’s task space. However, future experiments that go beyond a purely spatial analysis are clearly needed to determine the nature of the kinds of task-specific activity these data represent.

Discussion

In the present study, neural activity was recorded from the anterior cingulate cortex of C57Bl6/J mice to examine how such activity correlated with exploratory and recognition memory behaviors. Units were recorded in an empty arena, throughout the introduction of and subsequent habituation to the positions of two identical objects, and finally during tests of novel object and novel location recognition memory. The animals’ behavior during the tests was generally consistent with previous reports (Benice et al. 2006; Ennaceur and Delacour 1988; Ennaceur and Meliani 1992; Hammond et al. 2004; Powell et al. 2003), though an order effect was seen in the novel object
behavior following the test of location recognition. In general, activity of ACC neurons had no consistent spatial patterns in the open field, corroborating the findings of Hok and colleagues (2005), and was not modulated by running speed. The exception to this was the identification of “bulls-eye” cells which reliably fired more in the center of the cylinder. Analyzed by criteria similar to those employed by Granon and colleagues (2003), there was little compelling evidence for a relationship between the firing pattern and the rate of exploration. The addition of two identical objects elicited a considerable number of changes in neuronal activity around the object locations. Some neurons fired more strongly during object exploration, while others fired less; some were responsive to both objects, while others responded only to one; a sizeable proportion of neurons exhibited comparable responses across multiple sessions. The correlation between ACC neuronal activity and exploratory behavior was perhaps most striking during the tests of recognition memory. During the location recognition test, some neurons followed the familiar object to its new location, others fired exclusively where the object had been, and yet others fired to both current and former object locations. Finally, an enhanced discrimination was observed in the firing patterns of ACC neurons between the novel and familiar objects specifically in those animals showing the appropriate novelty-seeking behavior during the object recognition task. These data clearly demonstrate an association between ACC neuronal activation and object exploration, and more broadly are suggestive of a number of the functional roles associated with ACC contributing to the representation of salient features of the task.

The goal of the present study was not simply to demonstrate whether and how the ACC is involved in novel object and/or novel location recognition behavior, per se. In fact, the single lesion study in rodents addressing the question of such involvement (Ennaceur et al. 1997) does not support a critical role for this structure in either task. However, other studies in humans and primates lead one to expect neuronal correlates to at least some of the behaviors associated with these tests. Pihlajamäki and colleagues (2005; 2004) measured the BOLD response using fMRI and found greater activation in a range of temporo-occipital and frontal cortical areas, including the ACC, specifically during object recognition behavior. Lesion data from primate studies also suggest a role for the ACC in object recognition (Bachevalier and Mishkin 1986; but see Meunier et al. 1997). Involvement of the ACC in object recognition behavior may be viewed in the context of proposed dorsal and ventral processing streams for spatial and object information, respectively. Briefly, in this conception, the ventral visual stream processes information specific to the identity of the object, while the dorsal stream processes location information (Mishkin and Ungerleider 1982; Ungerleider et al. 1998; Ungerleider and Haxby 1994). The two, largely non-overlapping pathways are composed of
multiple structures with the ACC described as a component of the ventral pathway (e.g., Courtney et al. 1996; Young 1992). Therefore, these tests were utilized as a well characterized behavioral framework in which to examine the firing properties of individual ACC neurons in the freely behaving rodent in the absence of externally imposed rules. The ACC may best be described as functionally diverse, with evidence for simple motor and sensory information processing, as well as more cognitive phenomena such as memory, attention, novelty detection, and comparisons of expectation versus outcome. These data were collected to examine how the different hypothetical roles of ACC function may relate to the firing of its neurons during exploration and simple tests of memory.

In contrast to the ACC, other structures exhibiting task-related activation during object and/or location recognition in humans (Pihlajamaki et al. 2005; Pihlajamaki et al. 2004) have received considerable attention in rodents. One such structure is the parietal cortex. A component of the dorsal visual processing stream, the parietal cortex is preferentially active during novel location recognition (Pihlajamaki et al. 2005). Lesion studies in rats have demonstrated that the parietal cortex is involved in object exploration and the differential use of proximal and distal landmarks (Save et al. 1992a; Save and Poucet 2000; Save et al. 1992b). More recently, Save and colleagues (2005) demonstrated that neurons of the parietal cortex interact with the hippocampus to form spatial representations, with parietal neurons contributing to the incorporation of proximal landmarks (i.e., objects) in the spatial map. The involvement of the hippocampal formation in these recognition tests has been studied extensively. In humans (Pihlajamaki et al. 2004) the anterior hippocampal formation, perirhinal cortex, and anterior parahippocampal cortex during novel object recognition, and posterior hippocampal formation and posterior parahippocampal cortex during tests of novel location recognition. The processing of spatial information is associated more with the posterior (human) or dorsal (rodent) hippocampus. However, even within the dorsal hippocampus, individual sub-regions are differentially involved in the processing of spatial and non-spatial information (Hunsaker et al. 2007). Evidence for hippocampal involvement in object recognition has been reported, though it is unclear whether the evidence is attributable to the length of the delay to test (Clark et al. 2000; Hammond et al. 2004) or when the lesions are performed relative to the initial encounter (Gaskin et al. 2003). For both scenarios it was proposed that extra-hippocampal structures, such as the perirhinal cortex, mediate the behavior in the absence of the hippocampus, a possibility that is supported by lesion data (Kesner et al. 2001).

Though a specific role for parietal neurons in the encoding of proximal landmarks into spatial maps has been proposed (Save et al. 2005), evidence for “object-related” firing by hippocampal pyramidal neurons has also
been reported. Hippocampal place cells firing near a barrier placed in one environment can continue firing near that barrier in a completely different environment (Rivard et al. 2004), while place cells distal to the barrier “remap”, i.e. they fire in a completely unrelated manner (Muller and Kubie 1987). This suggests that, under certain circumstances, object-related information may be preserved across maps. However, the effects of object manipulations on place cells are qualitatively different from the results shown here for ACC neurons, as demonstrated by Lenck-Santini and colleagues (2005). When they moved familiar objects from their familiar positions the place cells that had firing fields near the objects would remap (i.e. they stopped firing or began firing in an unrelated fashion) rather than following the objects to their novel locations or continuing to fire to where the objects had been, as ACC neurons do in the Novel Location Task (Figure 6). Moreover, substituting a novel object for a familiar one (akin to the Novel Object Task) had no effect upon place fields regardless of where they were, even though the animals clearly showed behavioral bias to the novel object. As seen in Figure 7 this same manipulation causes many ACC neurons to significantly change their firing in the object locations. The most likely explanation is that the object-related firing of hippocampal place cells reflects the objects’ contribution to the spatial layout of the environment rather than the identity and position of the objects themselves. The remapping of place cells upon object translocation resembles that seen when spatial cues are rotated in opposition to each other (Tanila et al. 1997), and reflects the relative spatial salience of proximal cues to the animal (Knierim 2004). ACC neurons, however, appear to be largely without stable spatial correlates until objects are introduced, after which they tend to stably fire only in past and present object locations, and they clearly discriminate between objects. Thus, the correlates of ACC neurons appear to be more about the objects themselves than their contribution to environmental geometry. How these correlates relate to the prevailing hypotheses of ACC function is described below.

The ACC is interconnected with motor and sensory areas (e.g., Carmichael and Price 1995), and considerable evidence supports differential involvement of ACC sub-regions in the primate in motor-related functions (for review, see Isomura and Takada 2004). In many cases these functions are associated with goal-directed behaviors, which reflect higher order cognitive involvement in the motor behavior. For example, Kennerley and colleagues (2006) demonstrated that lesions of the ACC impair the ability of monkeys to consistently reproduce motor responses associated with a reward, though the motor response itself was intact. Subsequent work identified neural correlates in the ACC relating to decision variables of cost/benefit analysis, potential reward, and probability of success during performance of tasks with motor components relating to eye movement and lever presses.
The authors identified neurons in the ACC with a range of responses to one or more of the decision variables, and concluded that the data supported the hypothesis that the ACC is important to guiding goal-directed behavior and optimal decision making.

Recently, two studies in rats have highlighted the importance of taking into account behavioral (motor) variability in the interpretations of neuronal data collected during tests of working memory. Euston and McNaughton (2006) identified a positive correlation between the proportion of context-sensitive cells detected and the degree of path variability in an open field variant of the radial arm maze. Similarly, Cowen and McNaughton (2007) observed that subtle variations in head angle more readily explained changes in neuronal activity associated with the delay interval of a paired-associate discrimination task. In both studies, a higher proportion of cells exhibiting these responses were found in the dorsal anterior cingulate, a region neuroanatomically aligned more closely with the prefrontal cortex than the rest of the ACC (Jones et al. 2005) but receiving greater sensorimotor innervation than the more ventrally located prelimbic and infralimbic cortices (Hoover and Vertes 2007). In both studies, the authors suggest several possible explanations for these behavioral variations, including, though not limited to, coincident learning of the variation with the task rules or a means of enhancing task-relevant sensory input. They concluded that changes in activity recorded during the effective delay interval of the working memory tasks were not necessarily correlates of working memory, but were instead attributable to specific behaviors occurring during the delay interval.

There are, of course, motor and sensory components to object exploration (ocular control during approach, circumnavigating the object, nose-touches, whisking, etc.), and the possibility exists that some proportion of the firing correlates in these tasks reflect motor function. The data as they are presented here do not possess the requisite granularity of measurement of motor function to exclude such hypotheses. However, it is difficult to fully account for several observed firing patterns with a strict motor hypothesis. For instance, it is unclear why two identical objects in the familiar condition would consistently elicit different motor responses. It is also difficult to explain why some neurons that fire during exploration of an object will continue to fire in the location following object’s removal (e.g., Figure 6, Neuron 2), given that the search pattern and accompanying sensory inputs are presumably different in the two conditions. Finally, the motor hypothesis does not satisfactorily address why activity would change during exploration of a familiar object specifically during object recognition behavior (e.g., Figure 7, Neuron 2). Thus,
while motor correlates in these data cannot as yet be ruled out, many of the neuronal responses are difficult to explain by a purely motor hypothesis.

The object-related firing seen in anterior cingulate neurons is in some respects reminiscent of “object cells” observed in the inferior temporal (IT) cortex. While not firing strictly on a one-to-one basis to specific objects (Desimone et al. 1984), individual IT neurons do show preferences for specific objects (Chelazzi et al. 1998). IT neurons also reflect short-term memory, with sustained firing observed during a 3 second delay period following stimulus removal (Chelazzi et al. 1998). Yet several important differences exist between IT object cells and the ACC responses observed here. First, introduction of the novel object resulted in a more robust response by individual neurons to either the novel object or the familiar object. In contrast, the IT literature does not appear to provide a corollary for the increased neuronal preference for a familiar object. Second, the IT neuron correlate to short-term memory is distinct from the observation that some ACC neurons only become responsive to a location after the object has been moved (e.g., Figure 6, Neuron 1). Finally, during the Test Block, fully half of the neurons recorded begin showing sustained responses only after the first two sessions (i.e., only after the changes begin), indicating that the “changes”, including those associated with the novel location test [a manipulation that should not evoke differential responses in IT neurons (Lueschow et al. 1994)], effectively elicit specific changes in ACC neuronal activity.

Of course, given the large number of studies implicating the ACC in a variety of cognitive processes, one has to consider that the activity described here reflects cognitive aspects of the task. A role for the rodent ACC in memory processes has been proposed previously for both non-spatial (Frankland et al. 2004; Frankland et al. 2006; Rudebeck et al. 2007) and spatial (Teixeira et al. 2006) tasks. There are features of the firing patterns described here that could reflect aspects of the task recalled from memory. Novel object (and novel location) recognition behavior requires memory: a comparison between the two objects and the judgment that one has been experienced previously while the other has not. The present study details neuronal correlates of object recognition behavior as well as neuronal firing associated with locations where an object had been. While continued exploration of a previous object location is not typically reported for these tests, persistent exploration of previously salient locations has been reported (Save et al. 1992a), with both object additions and deletions effectively guiding visual system exploration (Brockmole and Henderson 2005). Together with observations that the same ACC neuron could also fire to the same object in the same location across multiple sessions, the data are suggestive of memory processes. However, the
present data do not distinguish between the ACC as a site of information storage or the online representation of information stored elsewhere in the brain (Bayley et al. 2005; Squire 1992; Squire and Zola 1996).

Two essential components of the present study relate to stimulus novelty and salience, judgments previously demonstrated to involve the ACC. Imaging data have implicated the ACC in novelty detection in humans (Downar et al. 2002; 2000), and exposure to novelty increases immediate early gene and associated mRNA expression in the ACC of rodents (Kabbaj and Akil 2001; Kabbaj et al. 2000; Montag-Sallaz et al. 1999; Papa et al. 1993). As was evident during Initial Exposure, the introduction of the objects to the arena elicited robust responses from a subpopulation of recorded neurons. The continued behavioral salience of the objects is clearly evident in the animals’ persistent exploration of those objects in subsequent sessions, despite their familiarity. At the neuronal level, one might expect that repeated exposure would result in habituation (Ranganath and Rainer 2003). However, salience at the neuronal level is reflected as well in the persistent firing of neurons during exploration of the familiar objects. This is in keeping with previous data indicating that, even after stimuli are no longer novel, they may continue to elicit ACC activation if they remain salient to the organism (Downar et al. 2003; Weible et al. 2003). The enhanced neuronal preferences observed during the behavioral expression of novel object recognition further reflect the sensitivity of the ACC to change.

One might expect that ACC neuronal responses to object novelty might more directly reflect the behavior, as they do other aspects of object exploration, such that cells would simply fire more strongly to the novel object versus the familiar. That object novelty evokes a transient change in neuronal response to both novel and familiar stimuli suggests that unexpected events trigger a change to each of the salient features of the environment, as the animal discriminates between the novel and familiar cues. This is consistent with proposed roles for the ACC in tasks such as set-shifting and task-switching. Both require the comparison of expected results, based on past performance, with actual outcomes, and the cognitive flexibility to shift behavior to new strategies to obtain a desired goal. While there is no “goal” in the object recognition task, beyond satisfying the innate drive to explore novel stimuli, the introduction of novelty does involve a deviation from the expected based on previous experience. In the rat, lesions of the ACC severely retard the intra-dimensional shift of a set-switching paradigm, during which distinctions between a new pair of the same class of cues (odor or digging media) is necessary for the shift in behavior (Ng et al. 2007). The ACC was proposed to mediate shifting attention between closely related task-relevant cues by decreasing attention to irrelevant stimuli, effectively increasing attentional control within the relevant
dimension. Task-switching requires the inhibition of a previously rewarded response in favor of a response that was previously unrewarded. In the primate, ACC neurons exhibit a robust, generalized increase in activity during the preparatory period of trials immediately following the switch. This elevated activation is transient, and decreases as the newly rewarded response is implemented with increasing success (Johnston et al. 2007). Novelty in the present study, representing a deviation from the expected, elicited a change in object responsiveness during the test of object recognition. The specificity of this effect in the ACC to object identity, and not location, may simply be by virtue of the neuroanatomy, reflecting the segregation of visual processing of spatial and non-spatial information into dorsal and ventral streams, respectively (Ungerleider and Haxby 1994).

Two recent studies (Fujisawa et al. 2008; Lapish et al. 2008) investigated network level changes in the prefrontal cortex and the ACC in freely behaving rodents. Both studies reveal specific, reproducible patterns of activity correlated with the animal’s behavior, and suggest that the aggregate of individual ACC neurons represent task space, though of course many of the same caveats described herein apply to their data as well. The present data are consistent with this conception. The focus here was exclusively on the activity of single neurons of the ACC, and as such provides a detailed view of the contents of the ensembles described by these investigators. The different correlates reflect different facets of the animals’ environment associated with both the open field (e.g., the bulls-eye cells) and the tests of recognition memory (e.g., either one or both objects, former object locations, and novel and familiar objects). The hypothesis that the ACC represents salient features of the task does perhaps best fit the present data, particularly if that representation is dynamic. In this way, not only are the salient features of the environment encoded, but changes to the environment are also tracked. As mentioned above, the specific impact of changes in object identity may be attributable to association of the ACC with the ventral visual processing stream. However, several lines of evidence from the present data suggest that there is a spatial component to identity. The dynamic representation of salient task features therefore would appear also to have the advantage that it includes aspects of many of the other functions ascribed to the ACC, as described above, increasing its utility as a conceptual framework for ACC function.

The present study describes, for the first time, single neuron correlates to object and location recognition behavior in the rodent ACC. These correlates include robust changes in activity associated with the exploration of objects, former object locations, and the introduction of novel objects into the environment. The observed patterns of activity are consistent with many of the processes associated with the ACC. The neuronal correlates of these distinct
processes may be elements of a more general, dynamic representation of salient task features, as has been proposed previously (Fujisawa et al. 2008; Lapish et al. 2008). These data confirm and extend the small but rapidly growing body of information relating to the firing patterns of neurons in the anterior cingulate cortex of freely behaving rodents by demonstrating how the introduction and manipulation of objects in an otherwise open field changes the kinds of information reflected by the firing of individual ACC neurons.


Knierrim JJ. How to avoid going bump in the night: object and place representations in the hippocampus. *J Gen Physiol* 124: 3-6, 2004.


Save E, Poucet B, Foreman N, and Buhot MC. Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behav Neurosci* 106: 447-456, 1992b.


Figure 1. Sequence of recording sessions throughout all task phases. Prior to tests of novel object (NO) or novel location (NL) recognition (Test Block), animals were familiarized to two identical objects in the same place for six days (the Familiar Condition, or FC). On the first day, neurons were recorded as animals explored the task environment (an open field, Open) without any objects, followed by the initial exposure (IE) to two identical objects. The Control Block sessions on day 6 were for comparison to the six Test Block sessions on day seven, measuring the variability in ACC neuronal firing patterns in six identical sessions.

Figure 2. Behavioral correlates of object novelty and location novelty. After six days of familiarization, tests of object and location recognition memory were performed serially and counterbalanced such that for one group of animals the novel object test preceded the novel location test (panels A-D) while the opposite was true for the other group (panels E-H). The circles in panels A and E represent the arena and the objects are represented as ObjectLocation #. The repeat sessions 2, 4, and 6 are behaviorally identical to sessions 1, 3 and 5, and are therefore not shown. A) One group of mice (n = 10) was tested first for novel object (session 3) and then novel location (session 5) recognition memory. The mice spent a similar amount of time exploring the two familiar objects in the familiar condition (A1 and A2; panel B). These mice exhibited a strong behavioral preference for both the novel object (B2; panel C) and the novel location (A3; panel D). E) The other group of mice (n = 7) was tested first for novel location (session 3), then novel object (session 5), recognition memory. These mice also spent a similar amount of time exploring the two familiar objects (panel F), and exhibited the expected preference for the novel location in the subsequent novel location recognition test (panel G). However, due presumably to an order effect and perseverative exploration of the former object location (A1), these animals failed to exhibit any behavioral preference for the novel object whatsoever (panel H). Novel stimuli are represented by black histograms, while familiar ones are represented by gray histograms, with * = p < .05.

Figure 3. Histological verification of electrode placement. A) Only data from tetrodes located in the ACC, extending from bregma rostral to the genu of the corpus callosum, were included in the present analyses. B) Photomicrograph of a tetrode track and termination (arrow) within the ACC, with the start of a second track, terminating in a more caudal section, visible on the dorsal cortical surface. The termination site corresponds to the
gray diamond illustrated (above) at coordinate 980µm anterior to bregma. cc: corpus callosum; Cg1: anterior cingulate gyrus region 1; Cg2: anterior cingulate gyrus region 2; gcc: genu of the corpus callosum; M2: secondary motor cortex. Dashed lines illustrate approximate boundaries between cytoarchitectonic regions. Illustrations adapted from Paxinos & Franklin 2001, 6th Ed.

Figure 4. Quantitative analysis of the firing patterns of anterior cingulate cortex (ACC) neurons before and after object addition. A) Information content, sparsity, coherence, and mean firing rate were essentially unaffected by the addition of objects to the open field (sessions 1 & 2 versus 3 & 4). B) Very little spatial correlation was observed between sessions 1 & 2 or sessions 2 & 3, confirming earlier results demonstrating a lack of spatial specificity in the firing of ACC neurons. However, the addition of objects caused many ACC neurons to modulate their firing around the object locations, leading to a significantly greater (p < .0001) overall spatial correlation in the subset of mice given a second Initial Exposure session (sessions 3 & 4), even without filtering for object-oriented cells.

Figure 5. The addition of objects to an open field changes the firing patterns of ACC neurons. Two neurons recorded from the same animal during the open field and initial exposure sessions illustrate two response types observed following the addition of the objects. Low information content, coherence, and correlation between Open Field sessions 1 & 2 indicate little spatial specificity in the activity of anterior cingulate cortex neurons prior to object addition. However, 13% (12/95) of neurons, referred to as “bull’s-eye” cells (e.g., Neuron 2), consistently exhibited higher firing rates toward the center of the arena. During Initial Exposure (session 3), 25% of neurons exhibited robust neuronal responses during object exploration (e.g., Neuron 1), with six of the former bulls-eye cells exhibiting reductions in firing rate in the vicinity of the objects. Although the animals spend more time at the object locations, the cells’ firing is not directly correlated with dwell time. This is illustrated by Neuron 1 where peak observed firing rates do not overlap with the occupancy map. Occupancy Maps and Rate Maps are auto-scaled for differences in rate across all 3 sessions. Greater dwell time (Occupancy Maps) is reflected by lighter pixels. Higher firing rates (Rate Maps) are reflected by dark red pixels, and lower rates by dark blue pixels. Firing rates reflect peak quintile rates of activity. The locations of the two identical objects are represented by “A”.

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Figure 6. **ACC neurons fire in all object locations during the novel location task.** These three neurons recorded from an animal given the novel location task prior to the novel object task typify the data obtained from this behavioral group. Object locations are given in the first column, the occupancy map is the second column, and the last three columns are firing rate maps of the cells. The occupancy map during session 3 illustrates the animal’s behavioral preference for the novel (A₃) versus familiar (A₂) location, as well as a tendency to continue exploring the now empty object location (A₁) from the Familiar Condition. This animal exhibited a small (non-significant) preference for the novel (B₂) versus familiar (A₃) object during session 5, but one can also see a much greater preference across sessions 3-5 for the location (A₁) where an object used to be in the Familiar Condition. Interestingly, Neuron 1 does not fire significantly at any object location until session 3, when it begins firing at location 1 after the object has been removed. Neurons 2 and 3 track the two objects across all six sessions, regardless of changes in location or identity. Additionally, Neuron 2 continues to fire in the former object location from the Familiar Condition during sessions 3, 5 and 6. Although the animals spend more time at the object locations, the cells’ firing is not directly correlated with dwell time. This is best illustrated by Neuron 3 where peak firing rates do not overlap with the occupancy maps throughout sessions 3-6. Occupancy Maps and Rate Maps are auto-scaled for differences in rate across all 6 sessions. In occupancy maps brighter pixels signify greater dwell time, while for rate maps higher firing rates are reflected by dark red pixels, and lower rates by dark blue pixels. Numeric firing rates (“X.XHz”) reflect peak quintile rates of activity.

Figure 7. **ACC neuronal responses to object novelty.** These two neurons recorded from an animal given the novel object task prior to the novel location task typify the data obtained from this group of mice demonstrating a clear behavioral response to object novelty. This animal’s marked behavioral preference continued through sessions 3, 4 & 6, although location novelty interferes with its expression in session 5. After firing to both object locations equally well, Neuron 1 exhibited a robust preference for the novel object’s location (B₂) upon its introduction in session 3. Neuron 2 exhibited the opposite preference, firing at the familiar object’s location (A₁) following introduction of the novel object at location 2, and even coming back during session 6. This preferential increase in mean firing rate at location 1 occurs even though the animal seldom visits the location, especially after the object itself is moved to location 3 in session 5. These neurons illustrate how even though the firing rate of some neurons (e.g. Neuron 1) strongly correlates with dwell time, many others (e.g. Neuron 2) do not: Neuron 2 peak firing rates
do not overlap with the occupancy maps throughout sessions 3-6. Occupancy Maps and Rate Maps are auto-scaled for differences in rate across all 6 sessions. Greater dwell time (Occupancy Maps) is reflected by lighter pixels. Higher firing rates (Rate Maps) are reflected by dark red pixels, and lower rates by dark blue pixels. Firing rates reflect peak quintile rates of activity.

**Figure 8. Novelty-seeking behavior correlates with enhanced object discrimination by ACC Neurons.**

Neurons are described as “preferring” one object over another when the response to the two objects differs significantly (unpaired t-test). A) For mice tested first for novel object recognition, in the Familiar Condition a comparable number of neurons exhibited an equally comparable preference for object A₁ (11 cells) or A₂ (7 cells). During the test of object recognition (session 3), more neurons preferred the novel (B₂; 22 cells) than the familiar (A₁; 14 cells) object ($\chi^2 = 4.57$). The preferences for objects B₂ and A₁ were comparable to each other, and significantly greater ($p<.05$) than preferences observed in the familiar condition. The preference for B₂ was still evident during session 5 (17 cells), and was greater ($df = 31, t = 2.239, p = .0325$) than the preference for A₃ (16 cells) and those observed during session 1 ($p<.05$). B) When location recognition behavior was tested for first, comparable numbers of neurons preferred either object A₁ (10 cells) or A₂ (6 cells) in the Familiar Condition, A₃ (9 cells) or A₂ (8 cells) during the location recognition test, and A₃ (12 cells) or B₂ (12 cells) during the object recognition test. All preference magnitudes were comparable, and all were significantly less ($p<.05$) than the preferences exhibited when object recognition behavior was tested for first for objects A₁ and B₂ during session 3, and object B₂ during session 5. The site of novelty is represented in black, while the familiar is represented in gray, but note that novelty is relative: the “familiar” site of sessions 5 & 6 was the “novel” site of session 3 & 4, and both behavioral and neuronal correlates appear to persevere (e.g. the preference seen for the “familiar” object location in panel C, which contains the novel object).

**Figure S1. Spiking data were analyzed only when corresponding clusters were clearly separable from surrounding activity.** Behavioral (positional) and neuronal data were recorded (Neuralynx, Bozeman, MT) as mice explored the cylindrical arena. In the above example (Neuron 1 from Figure 5), the Occupancy Maps illustrate the behavior of a mouse during exploration of two objects in the Familiar Condition, followed by exploration of a Novel Object (sessions 3 & 4) and a Novel Location (sessions 5 & 6). Neuronal data collected simultaneously are analyzed off-line using cluster cutting software MClust (A.D. Redish, University of Minnesota). The activity of
individual cells are isolated using 2-dimensional plots generated from pairs of waveform measures (peak height voltage, valley voltage, and energy) taken from the wires of a single tetrode. The waveforms corresponding to clearly separable clusters (e.g., the “blue” cluster of points in the Cluster Plots) are then generated (Waveforms). The spike data corresponding to each cluster are correlated with the positional data to generate Spike Maps illustrating the neuron’s activity in 2-dimensional space, as well as the mean firing and peak quintile firing rates (Firing Rates).

\textbf{Figure S2. Rate maps illustrate low and high examples from the range of spatial information, sparseness, and coherence values observed in the open field.} A) Rate maps are illustrated for neurons exhibiting low and high values for measures of spatial information, sparseness, and coherence. The value for each measure is given beneath the respective rate map. The firing rate for each neuron is included to the right of each rate map. B) Univariate scatter plots illustrate the distribution of values for spatial information, sparseness, and coherence. The bivariate scatter plot illustrates the relationship between measures of spatial information and coherence.
<table>
<thead>
<tr>
<th>Days of Training</th>
<th>Sessions</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Familiarization</td>
<td>Initial Exposure</td>
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<tr>
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<td>Re-Exposure</td>
<td>FC</td>
<td></td>
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<tr>
<td></td>
<td>Re-Exposure</td>
<td>FC</td>
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<td></td>
<td>Re-Exposure</td>
<td>FC</td>
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<td>Re-Exposure</td>
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</tr>
<tr>
<td></td>
<td>Control Block</td>
<td>FC</td>
<td>FC</td>
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<td>NO</td>
<td>NL</td>
<td>NL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FC</td>
<td>FC</td>
<td>NL</td>
<td>NL</td>
<td>NO</td>
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</tr>
<tr>
<td>Sessions</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
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<td>-------</td>
<td>-------</td>
<td>-------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Information</td>
<td>.79 ± .09</td>
<td>.85 ± .14</td>
<td>.74 ± .11</td>
<td>.79 ± .17</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sparseness</td>
<td>.57 ± .03</td>
<td>.57 ± .03</td>
<td>.59 ± .02</td>
<td>.60 ± .04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coherence</td>
<td>.21 ± .02</td>
<td>.19 ± .02</td>
<td>.25 ± .02</td>
<td>.25 ± .03</td>
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<td></td>
</tr>
<tr>
<td>Mean Rate</td>
<td>4.10 ± .70</td>
<td>3.82 ± .62</td>
<td>4.73 ± .62</td>
<td>5.08 ± .73</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Diagram:**

- **Correlation Scores**
- **Session Pairs**
- 45 cells
- 95 cells
- 45 cells

**Legend:**
- 95 cells
- 95 cells
- 45 cells
Occupancy Map

Rate Maps
Neuron 1 Neuron 2

Session 1
Object Locations
Occupancy Map

2.3Hz 15.0Hz

Session 2

1.8Hz 10.6Hz

Session 3
Object Locations

A1 A2

6.0Hz 8.3Hz

Neuron 1 Neuron 2
Session 1: Familiar Condition
- Object Locations: A1, A2
- Neuron 1: Rate Maps: 6.0Hz, 7.7Hz, 12.0Hz
- Neuron 2: Rate Maps: 7.0Hz, 7.3Hz, 7.5Hz
- Neuron 3: Rate Maps: 7.0Hz, 7.3Hz, 7.5Hz

Session 2: Familiar Condition
- Object Locations: A1, A2
- Neuron 1: Rate Maps: 6.0Hz, 7.7Hz, 12.0Hz
- Neuron 2: Rate Maps: 7.0Hz, 7.3Hz, 7.5Hz
- Neuron 3: Rate Maps: 7.0Hz, 7.3Hz, 7.5Hz

Session 3: Novel Location
- Object Locations: A2, A3
- Neuron 1: Rate Maps: 14.6Hz, 8.5Hz, 6.3Hz
- Neuron 2: Rate Maps: 12.9Hz, 7.6Hz, 5.9Hz
- Neuron 3: Rate Maps: 17.6Hz, 7.1Hz, 8.0Hz

Session 4: Novel Location
- Object Locations: A2, A3
- Neuron 1: Rate Maps: 14.6Hz, 8.5Hz, 6.3Hz
- Neuron 2: Rate Maps: 12.9Hz, 7.6Hz, 5.9Hz
- Neuron 3: Rate Maps: 17.6Hz, 7.1Hz, 8.0Hz

Session 5: Novel Object
- Object Locations: B2, A3
- Neuron 1: Rate Maps: 21.1Hz, 7.5Hz, 8.6Hz
- Neuron 2: Rate Maps: 7.1Hz, 8.0Hz
- Neuron 3: Rate Maps: 7.5Hz, 8.6Hz

Session 6: Novel Object
- Object Locations: B2, A3
- Neuron 1: Rate Maps: 21.1Hz, 7.5Hz, 8.6Hz
- Neuron 2: Rate Maps: 7.1Hz, 8.0Hz
- Neuron 3: Rate Maps: 7.5Hz, 8.6Hz

auto-scaled maps
Preference Magnitude (z-scores)

Session 1
- Session 3
- Session 5

Familiar Condition

Novel Object

Novel Location
Table 1. Object types and dimensions.

<table>
<thead>
<tr>
<th>Object</th>
<th>Dimensions (L x W x H cm)</th>
<th>Area (sq. pixels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper Clip Tray</td>
<td>9.5 x 5.5 x 3.0</td>
<td>9.8</td>
</tr>
<tr>
<td>Business Card Tray</td>
<td>9.5 x 6.0 x 4.2</td>
<td>10.7</td>
</tr>
<tr>
<td>Rubber Door Stop</td>
<td>12.0 x 5.5 x 3.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Gray Lego Object</td>
<td>8.0 x 4.5 x 4.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Black Lego Object</td>
<td>8.0 x 4.5 x 5.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Blue Lego Object</td>
<td>9.5 x 4.5 x 3.8</td>
<td>8.2</td>
</tr>
</tbody>
</table>
Table 2. Proportions of cells responding during training

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Cells</th>
<th>All Sessions</th>
<th>Per Session</th>
<th>Sust</th>
<th>RI</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Exposure</td>
<td>11</td>
<td>95</td>
<td>24 (25%)</td>
<td>24 (25%)</td>
<td>23%</td>
<td>18%</td>
<td>5%</td>
</tr>
<tr>
<td>Control Block</td>
<td>12</td>
<td>134</td>
<td>95 (71%)</td>
<td>16 (12%)</td>
<td>36%</td>
<td>24%</td>
<td>12%</td>
</tr>
<tr>
<td>Novel Location First</td>
<td>7</td>
<td>104</td>
<td>97 (93%)</td>
<td>16 (15%)</td>
<td>42%</td>
<td>30%</td>
<td>12%</td>
</tr>
<tr>
<td>Novel Object First</td>
<td>10</td>
<td>119</td>
<td>102 (86%)</td>
<td>17 (14%)</td>
<td>38%</td>
<td>28%</td>
<td>10%</td>
</tr>
</tbody>
</table>

Cells: # of cells; N: # of animals; RD: rate decreasing, sustained; All Sessions: total object responsive; RI: rate increasing, sustained; Per Session: total object responsive divided by # of sessions; Sust: sustained responses.