

Role for Cingulate Motor Area Cells in Voluntary Movement Selection Based on Reward

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Most natural actions are chosen voluntarily from many possible choices. An action is often chosen based on the reward that it is expected to produce. What kind of cellular activity in which area of the cerebral cortex is involved in selecting an action according to the expected reward value? Results of an analysis in monkeys of cellular activity during the performance of reward-based motor selection and the effects of chemical inactivation are presented. We suggest that cells in the rostral cingulate motor area, one of the higher order motor areas in the cortex, play a part in processing the reward information for motor selection.

The cingulate motor areas (CMAs) of primates reside in the banks of the cingulate sulcus in the medial surface of the cerebral hemisphere and are subdivided into rostral and caudal parts (1). Anatomical studies have revealed prominent afferent input to the

CMAs from the limbic structures and the prefrontal cortex, which can send information about motivation and the internal state of subjects, as well as cognitive evaluation of the environment (2, 3). The CMAs send output to the primary and secondary motor areas

and other motor structures in the brainstem and spinal cord (4). The CMAs are thought to be in a pivotal position to process the information necessary to select voluntary actions in accordance with the subject's internal and external requirements because of this anatomical connectivity (5-7). However, it is not yet known exactly how the CMAs are used or how individual cells behave in relation to the actual performance of motor tasks requiring motor selection based on reward evaluation. Therefore, we devised an experimental model of reward-based motor selection and analyzed cellular activity in the CMAs.

We trained three monkeys (*Macaca fasciata*) to perform two different arm movements, either pushing or turning a handle, in response to a visual trigger signal (8, 9). The essence of the motor task was that the animals voluntarily selected one of the two movements based on the amount of reward.

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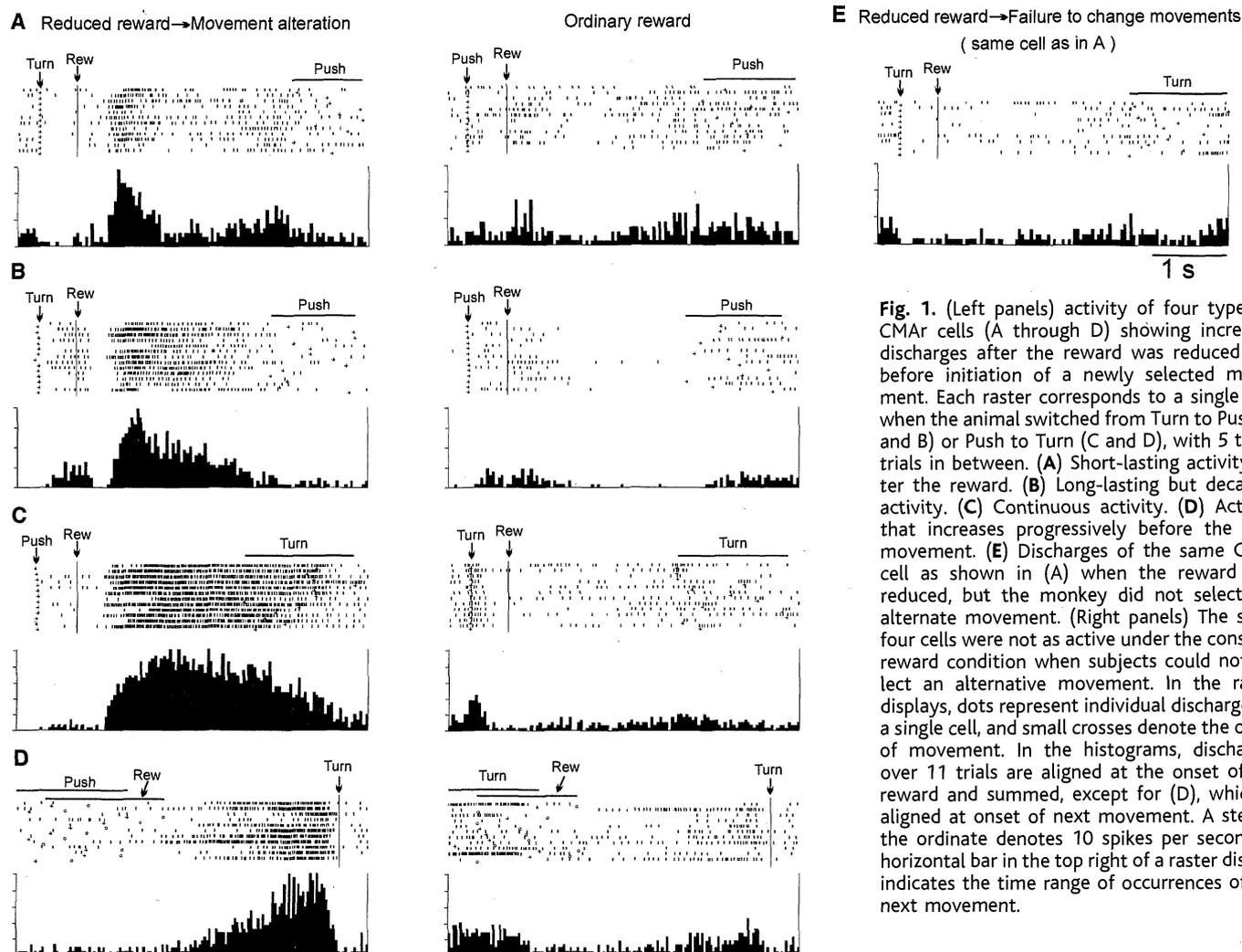


Fig. 1. (Left panels) activity of four types of CMA cells (A through D) showing increased discharges after the reward was reduced and before initiation of a newly selected movement. Each raster corresponds to a single trial when the animal switched from Turn to Push (A and B) or Push to Turn (C and D), with 5 to 12 trials in between. (A) Short-lasting activity after the reward. (B) Long-lasting but decaying activity. (C) Continuous activity. (D) Activity that increases progressively before the next movement. (E) Discharges of the same CMA cell as shown in (A) when the reward was reduced, but the monkey did not select the alternate movement. (Right panels) The same four cells were not as active under the constant reward condition when subjects could not select an alternative movement. In the raster displays, dots represent individual discharges of a single cell, and small crosses denote the onset of movement. In the histograms, discharges over 11 trials are aligned at the onset of the reward and summed, except for (D), which is aligned at onset of next movement. A step in the ordinate denotes 10 spikes per second. A horizontal bar in the top right of a raster display indicates the time range of occurrences of the next movement.

During a series of constant-reward trials, they kept selecting a particular movement. If the reward was reduced, they chose to perform the alternate movement. We used conventional single-cell recording techniques to record cellular activity from the rostral and caudal cingulate motor areas [CMAr and CMAc; see (10)], as well as from the primary motor area. We confirmed the cortical recording sites on the basis of histological and physiological criteria (1, 7, 10). As reported previously, the CMAr and CMAc are found in the upper and lower banks, and their transitional areas, of the cingulate sulcus. The neurons described here were recorded at sites in the CMAs that (i) project to the primary motor cortex (11) and (ii), when stimulated, evoke limb movements (12).

We found that four types of cells in the CMAr exhibit changes in activity during the interval between the occurrence of the reduced reward and the initiation of a new selected movement. The first type of cell had phasic activity that began 200 to 600 ms after the occurrence of the reduced reward, and well before the monkey initiated the alternate movement for the next trial, as shown in Fig. 1A (left panel). The same cell did not respond to the reward as long as the reward was not reduced (right panel). The second type showed a long-lasting change in activity (>1000 ms following the reduced reward; Fig. 1B, left) that also built up rapidly, but decayed before initiation of the next movement. The third type had a rapid build-up in activity starting more than 200 ms after the reduced reward, with little decay before initiating a new movement on the basis of the reduced reward (Fig. 1C). The fourth type showed a more gradual increase in activity that peaked near the initiation of the movement selected after the reduced reward (Fig. 1D). Interestingly, none of these types of cellular activity was observed when the reward was reduced, but the monkey did not select the alternate movement (Fig. 1E). These four types of cells appear useful in relaying information about the reward reduction to the process of selecting the next movement, at least in the simplified model of motor selection we present.

In the CMAr, cellular activity of the types described above (selective relation to reduced reward/motor selection) was found in 81 (37%) of 221 task-related cells in the CMAr (Table 1) (13). A prominent property of these cells was that the majority ($n = 55$, or 68%) of the activity depended on which of the two movements the subjects selected, and thus was differential [$P < 0.01$; (14)]. A typical example of the differential activity is shown in Fig. 2. The cell is active after the reduced reward (Fig. 2A), but the increase in activity is selective to trials when the monkey selected Turn as the next movement (right panels),

changing from the previous movement of Push, but not when the change was the reverse (left panel) (15).

To test the possibility that the cellular activity may be related nonspecifically to a signal requiring the animal to change future movements, rather than specifically to the reward information, we added a control task. For the control task, a tone signal (1 kHz, 300 ms) in the waiting period told the animal to change the future movements, thereby impos-

ing animals to select an alternative movement involuntarily. The cell shown in Fig. 2C (same cell as in Fig. 2A) did not respond to the tone signal, even when the animal changed from Push to Turn (right). The same test was performed while recording from 46 CMAr cells that responded to the reduced reward. A great majority ($n = 41$) responded selectively to the reduced reward rather than to the tone signal, indicating a preferential relationship between the CMAr cells and the

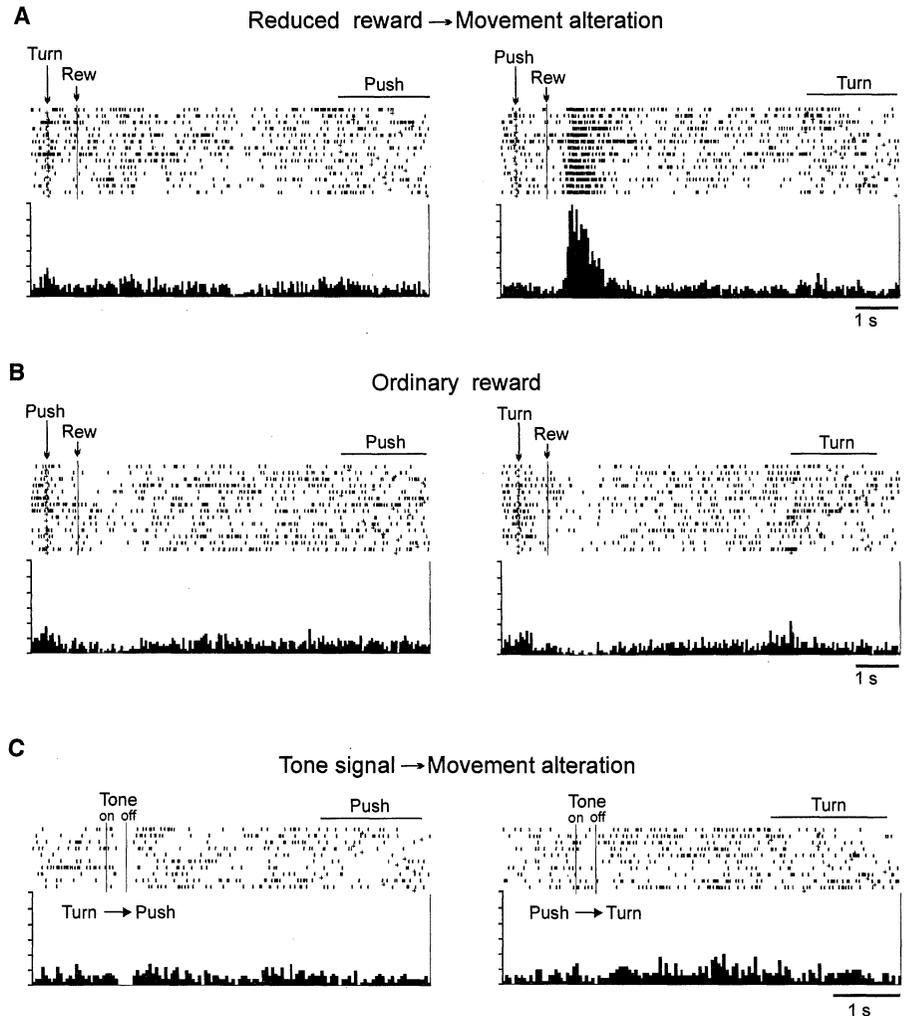


Fig. 2. (A) Activity of a CMAr cell exhibiting an increase in discharge after the occurrence of the reduced reward, but only when the monkey selected the Turn movement after changing from the Push movement (right), and not when the next selection after the reduced reward was Turn (left), or (B) when the reward remained unchanged. (C) The same cell did not respond to the tone signal that instructs the animal to change movements.

Table 1. Distribution of cells in CMAr and CMAc related to different aspects of motor task. Prep., preparatory; Pre-, premovement; Post-, postmovement.

Cingulate motor area	Selective relation to reduced reward/movement selection				Prep.	Pre-	Post-	Reward	Total
	Type 1	Type 2	Type 3	Type 4					
CMAr	33	22	16	10	74	38	12	16	221
CMAc	0	1	0	3	34	63	17	2	117

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reward-based motor selection. In the remaining cells, however, the response was nonselective. These cells may be involved in the shift of future movements, much as cells reported in the presupplementary motor area (16).

In the CMAR, other types of cells showed activity related to movement initiation ($n = 38$), the period of preparation for the next movement or movements ($n = 74$), or the occurrence of the reward nonselectively (constant or reduced, $n = 16$). In contrast, the activity of cells in the CMAc was mostly related to movement initiation or motor preparation. Only four CMAc cells showed activity related to motor selection (Table 1). The proportion of cells that were active in motor

selection was significantly less in the CMAc than in the CMAR ($P < 0.001$ by chi-square test). In the primary motor cortex, we analyzed the activity of 114 task-related cells; none exhibited the properties shown in Fig. 1 or 2 (17). The premovement preparatory activity was slightly dissimilar ($P < 0.05$) in only two cells, depending on whether the subject selected the alternate movement.

If the CMAR is crucially involved in the process of reward-based motor selection, then deactivation of this area is likely to impair the ability to select an appropriate movement. This was exactly what we observed by reversible inactivation of the CMAR with topical application of muscimol, a γ -aminobutyric acid agonist (18). When 3

to 4 μl of muscimol was injected bilaterally in the forelimb part of the CMAR, the monkey began to fail to select a correct movement 10 to 15 min after injection. Even if the reward was reduced considerably, the monkey kept selecting the previously performed movement and failed to select the alternate movement (Fig. 3). At other times, the animal made a mistake and prematurely selected the alternate movement before the reward was reduced. These effects, observed at six injection sites in the forelimb part of the CMAR, were dose-dependent and not observed with a concentration of less than 5 $\mu\text{g}/\mu\text{l}$. The effects were not observed when we injected muscimol bilaterally into the hindlimb representation area of the CMAR or into the forelimb part of the CMAc. Furthermore, it was confirmed in four additional injection experiments that the animal had no problems in selecting the alternative movements when the alteration was cued with the tone signal (19), despite the failure in the reward-based motor selection.

Previous brain-imaging studies in humans have shown that the region presumably corresponding to the CMAR is particularly active when a variety of motor tasks require subjects to voluntarily select movements (7, 20). Our findings determined that the CMAR is indeed crucially involved in voluntary motor selection. Our study also indicates that the CMAR contains cells active in relation to motor selection based on the amount of reward. Such cells seem to be profoundly involved in processing information about assessing the reward obtained by executing a current movement and selecting the next movement if the reward is not satisfactory. Anatomical studies have revealed connections from the amygdala and ventral striatum to the anterior cingulate cortex (area 32) and the cingulate gyrus (21), which in turn project to the CMAR (2, 3). These limbic projections provide ample information about reward values that are directly connected to the goals of motor acts. On the other hand, the direct or indirect pathways from the prefrontal cortex to the CMAR (2, 3) are able to transmit information concerning short-term memory about the occurrence of events during the performance of a motor task in the previous trial (22). Thus, the CMAR is ideally situated to combine information from the two sources, and our results suggest a way in which CMAR cells make use of the combined information to select an appropriate motor act.

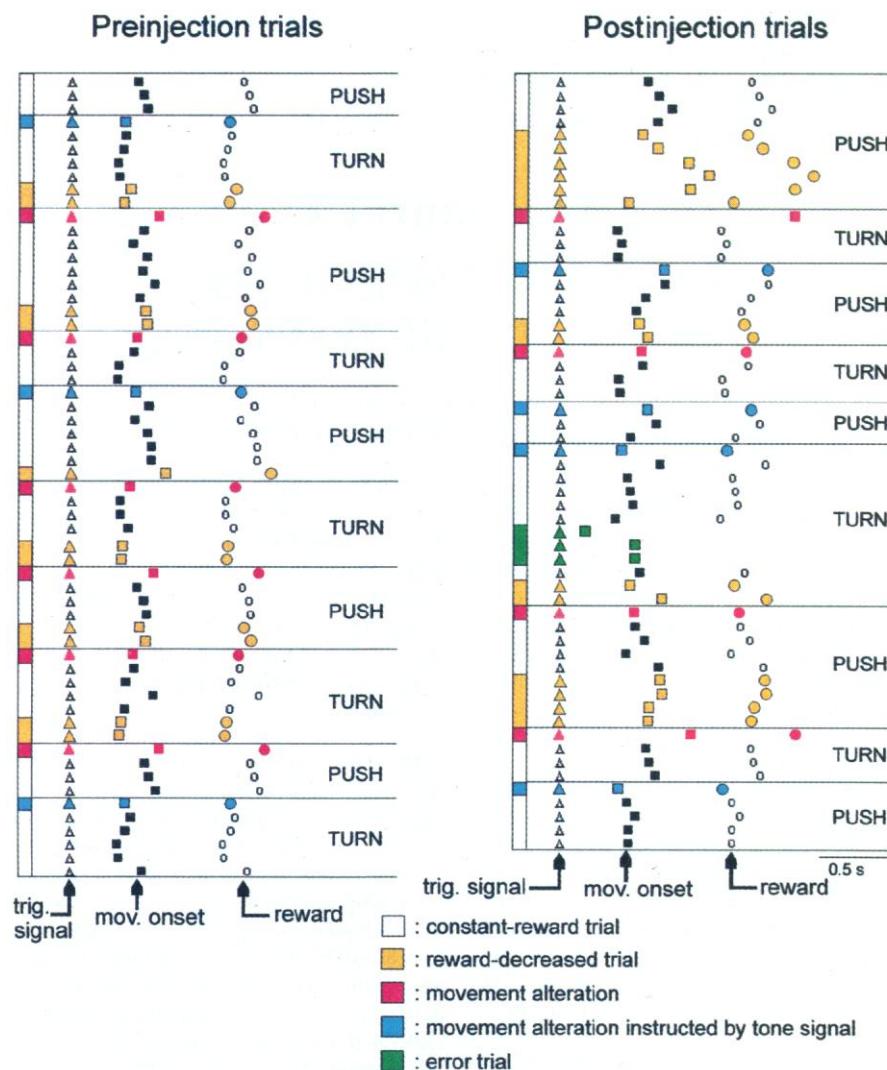


Fig. 3. Effects of muscimol injection into the CMAR on the task performance visualized with a serial display (from top to bottom) of the time course of consecutive performance of either Push or Turn movement, with each row representing individual trials. Triangles, squares, and circles indicate the onsets of trigger signals, movements, and reward delivery, respectively. After completing each movement, the handle was quickly returned to the hold zone mechanically, where the monkey had to hold it until the next trigger signal appeared. After the muscimol injection (right), the animal often failed to select the alternate movement despite a considerable reward decrease (yellow symbols) or made a mistake of selecting the alternative movement too early, before the reward decrease (green symbols).

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8. The animals were cared for in accordance with NIH's *Guide for Care and Use of Laboratory Animals*, and *Guidelines for Institutional Animal Care and Use* published by Tohoku University School of Medicine.

9. Behavioral task: When the monkey held the handle in a neutral position for 2.8 to 7.8 s, an LED (light-emitting diode) was illuminated as a signal to start the correct movement. Initially, the subject had to guess which of the two choices was correct. Performing the correct movement was rewarded with a drop of fruit juice, and the correct movement remained unchanged in a block of trials, so that the monkey was required to keep selecting the same movement. The amount of the reward remained constant (0.1 ml) for four to 12 successive trials, unless the subject made a mistake and selected the wrong movement, in which case an audible warning tone replaced the reward. Subsequently, the amount of the reward decreased by 30% for each correct trial. At this stage, monkeys were free to select the alternate movement. They usually did so after the first to the third decrement (30 to 65.7% decrease in reward). If they did, the alternate movement was then defined as the correct movement, the reward reverted to the full amount, and a new series of constant-reward trials began, with the redefined correct movement.

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11. After completion of recording cellular activity, recording sites were marked with microlesions (by passing small currents through recording electrodes). Thereafter, the primary motor cortex was carefully mapped with intracortical microstimulation. A chemical tracer, wheat-germ agglutinin-horseradish peroxidase (WGA-HRP, 0.2 μ l), was injected into four sites within both the proximal and distal forelimb areas of the primary motor cortex. In the recording sites in the CMAs, HRP-positive cells were found with a standard technique (10). Because we found no spread of injected WGA-HRP into the face area, we concluded that the portions of CMAr and CMAc from where we recorded cellular activity coincided with the areas that projected to arm representation areas of the primary motor cortex.

12. Intracortical microstimulation was delivered with 20 to 40 pulses of 0.2-ms duration at 333 Hz at 20 to 50 μ A. Responses of cells to somatosensory stimulation (responses to joint manipulations or stroking the skin) also helped to find that the recording sites were forelimb representation areas and not face or neck areas.

13. Neuronal activity was quantitatively analyzed after constructing a peri-event histogram by summing the data from at least 10 trials for each condition. We defined the activity as task-related when the number of discharges in at least three successive 20-ms bins of the peri-event histogram during the four task periods deviated from the mean value during a control period by more than 2 SDs. The task periods included preparatory (from the time the handle was held in the central position to the trigger signal), premovement (from the trigger signal to the onset of movement), postmovement (from the onset of movement to delivery of the reward, 400 ms after the execution of the movement), and reward (500 ms after reward delivery). The control period was the last 500 ms of the intertrial interval. We performed analysis of variance (ANOVA) to test whether the activity in the reduced reward condition differed from that in the ordinary reward condition for the data obtained on a trial-by-trial basis.

14. We performed ANOVA to test this difference.

15. This finding suggests that the activity of these selective cells influences a motor decision process (facili-

tating selection of one movement and suppressing the other), rather than reflecting an affective aspect of the reward.

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17. The neurons were recorded from both the proximal (arm) and distal (hand) representation areas in the primary motor cortex, defined by the effects of intracortical microstimulation. In addition to this study on the 114 neurons in these areas, we also examined neuronal responses in the neck, back, and shoulder representation areas. In these areas, we did not observe the selective responses appearing after the reduced reward. We also analyzed extensively the activity in the neck and back muscles (with electromyogram) and found no responses appearing selectively after the reduced reward.

18. After completing the cellular recording sessions, we inserted small-caliber (300 μ m) injection cannulae bilaterally into the CMAs and injected a small amount (2 to 4 μ l, 1 to 10 μ g/ μ l, 1 μ l in 10 min) of muscimol to evaluate the effects of temporary deactivation of each area. The least effective concentration was 5 μ g/ μ l.

19. The reaction time (defined as the interval between the beginning of the "start" signal and the movement onset) and movement time (the interval from the movement onset to the attainment of the required motor-target) during performance of the motor task were always analyzed. When the movement alter-

ation was cued with the tone signal, neither values was found to be significantly different when compared before and after the muscimol injection ($P > 0.1$ by Mann-Whitney U test). This means that the animals' ability to execute the movements per se was not much influenced by the muscimol application. However, after the muscimol injection, the reaction time was lengthened ($P < 0.01$) when the animal selected the alternate movement in response to the reduced reward, although the movement time was not lengthened ($P > 0.05$).

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Control of Neonatal Tolerance to Tissue Antigens by Peripheral T Cell Trafficking

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Self tolerance is acquired by the developing immune system. As reported here, particular properties of the neonatal tissue contribute to this process. Neonatal skin, but not adult skin, was accessible for naïve CD8 T cells. In mouse bone marrow chimeras generated at different ages, recent thymic emigrants were tolerized to a skin-expressed major histocompatibility complex class I antigen only during a neonatal period but not during adulthood. Blockade of T cell migration neonatally prevented tolerance induction. Thus, T cell trafficking through nonlymphoid tissues in the neonate is crucial for the establishment of self tolerance to sessile, skin-expressed antigens.

Differences in tolerance induction during the neonatal and adult periods of life have fascinated immunologists since the pioneering work of Billingham, Brent, and Medawar (1). Neonatal mice, in contrast to adults, develop lifelong tolerance to allogeneic skin grafts when exposed to allogeneic cells of the same donor strain; hence, self tolerance is actively acquired. The newborn immune system can also mount an immune response when chal-

lenged (2). Thus, there appear to be quantitative but not qualitative differences among the cells generating an immune response (2).

Although these investigations have focused on systems in which mobile antigen-presenting cells pick up antigen and carry it to lymphoid organs for T cell recognition, the role of differential T cell migration in tolerance induction to sessile self antigens expressed exclusively on extrathymic tissues is undefined. Large-scale trafficking of virgin T cells through extralymphoid tissues has been observed in fetal sheep, in contrast to the restricted circulation in the adult animal (3). To test whether differential T cell migration through neonatal versus adult tissue would influence tolerance induction to tissue-specific self antigens, we used a transgenic mouse model expressing the major histocompatibility complex (MHC) class I antigen K^b under

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