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Super-Flinchers and Nerves of Steel: Defensive Movements Altered by Chemical Manipulation of a Cortical Motor Area

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Summary

In a restricted zone of the monkey motor cortex, neurons respond to objects near, approaching, or touching the body. This polysensory zone was hypothesized to play a role in monitoring nearby stimuli for the guidance of defensive movements. To test this hypothesis, we chemically manipulated sites within that zone by injecting bicuculline (increasing neuronal activity) or muscimol (decreasing neuronal activity). Bicuculline caused the monkey to react in an exaggerated fashion to an air puff on the face and to objects approaching the face, whereas muscimol caused the monkey to react in a reduced fashion. The effects were expressed partly as a motor abnormality (affecting movement of the musculature contralateral to the injection site) but also partly as a sensory enhancement or sensory neglect (affecting responses to stimuli contralateral to the injection site). These findings suggest that the polysensory zone contributes to the ethologically important function of defense of the body.

Introduction

How does the primate brain protect the body from nearby, threatening objects? In monkeys, a polysensory region in the precentral gyrus may participate. Neurons in this region respond to tactile, visual, and sometimes auditory stimuli (Fogassi et al., 1996; Gentilucci et al., 1988; Graziano and Gandhi, 2000; Graziano et al., 1997, 1999; Rizzolatti et al., 1981). These polysensory neurons were first reported in ventral area 6, or the ventral premotor cortex (PMv), shown in Figure 1A (Graziano et al., 1997; Rizzolatti et al., 1981). Their location was specified further to a posterior part of PMv termed F4, shown in Figure 1B (Gentilucci et al., 1988; Matelli et al., 1985). In a recent mapping study, the polysensory neurons were found to be clustered in a region that may roughly match the dorsal half of F4 (Figure 1C), though the size and exact location of this polysensory region varies somewhat among monkeys (Graziano and Gandhi, 2000). We refer to this region of distinct multimodal sensory properties as the polysensory zone (PZ).

Neurons in PZ respond to stimuli near, approaching, or touching the head, torso, or arms (Rizzolatti et al., 1981; Gentilucci et al., 1988; Fogassi et al., 1996; Graziano et al., 1997, 1999; Graziano and Gandhi, 2000). The same neurons are active during defensive behavior, such as during an air puff to the cheek, and the activity of the neurons correlates with the magnitude of the defensive reaction (Cooke and Graziano, 2004). Electrical microstimulation in PZ in awake or anesthetized animals evokes defensive-like movements (Graziano et al., 2002a, 2002b; Cooke and Graziano, 2004) that match in detail normal defensive reactions to an air puff (Cooke and Graziano, 2003). These movements include a squint and blink; lifting of the upper lip; flattening of the ear against the head; turning away of the head; shrugging of the shoulder; in especially strong reactions, such as in the first few trials of a block of air puff trials, a lifting of the hand into the space near the head; and a defense-related, nonsaccadic centering of the eyes.

These results, especially the effect of electrical stimulation, argue for a role of PZ in the control of defensive movements. However, these previous studies leave several questions unanswered. Electrical stimulation affects both cell bodies and fibers of passage and can result in both orthodromic and antidromic signal transmission. Would other types of stimulation that do not affect fibers of passage or result in antidromic stimulation have a similar effect? Would modulating the level of neuronal activity in PZ lead to changes in the monkey's ability to make actual defensive movements in reaction to threatening stimuli? Is neuronal activity in PZ necessary for normal defensive movement? To address these questions, in the present experiment we tested the effect of direct chemical manipulation of the neuronal activity in PZ on the monkey's defensive reactions. Specifically, we measured the defensive reaction to an air puff while reversibly inhibiting sites in PZ with the chemical muscimol or reversibly disinhibiting sites with bicuculline.

A defensive reaction to a sudden stimulus such as an air puff has two phases. It begins with a fast, stereotyped startle that is thought to be subcortically mediated (Strauss, 1929; Landis and Hunt, 1939; Koch, 1999; Yeomans et al., 2002). The startle is not spatially directed; it is typically bilaterally symmetric, including, for example, a bilateral blink. The second phase of the reaction is more sustained and directed toward the spatial location of the threat, for example, a squint that is stronger on one side of the face or a blocking movement of the arm (Strauss, 1929; Landis and Hunt, 1939; Schiff et al., 1962; King et al., 1992; Cooke and Graziano, 2003). Electrical stimulation of PZ evokes sustained, spatially directed movements that resemble the second phase of a defensive reaction; therefore, we hypothesized that PZ may participate in this second phase, encoding the locations and trajectories of nearby objects and coordinating an appropriate defensive response (Cooke and Graziano, 2003, 2004). In this hypothesis, chemical manipulation of PZ should affect the second phase of a defensive reaction and have little effect on the initial startle response.

Results

Effect of Chemical Injection on Neuronal Activity

To ensure that bicuculline and muscimol did actually alter the level of neuronal activity at the site of cortical injection, we injected the drug while simultaneously

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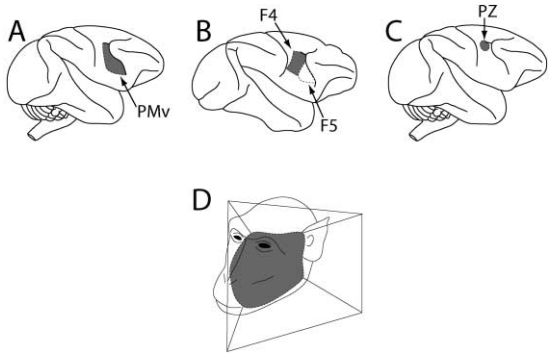

Figure 1. Location and Sensory Properties of the Polysensory Zone

(A) The ventral premotor cortex (PMv), in which polysensory neurons were reported (e.g., Graziano et al., 1997).

(B) Polysensory neurons were found to be concentrated in area F4 (Gentilucci et al., 1988; Matelli et al., 1985).

(C) The approximate location of the polysensory zone (PZ) as determined in a mapping study by Graziano and Gandhi (2000).

(D) Tactile receptive field (shaded) and visual receptive field (boxed area extending from shaded area) of a typical bimodal, visual-tactile neuron in PZ.

measuring the ongoing neuronal activity using a Crist recording microsyringe. Although the microsyringe electrode had a low impedance (0.5 MΩ), and therefore single neurons could not easily be isolated, it did allow for the measurement of background multiunit activity, which was sufficient to test the effect of the injected drug.

Figure 2A shows the effect of muscimol injection (10 μg in 1 μl of saline, injected at 0.1 μl/min) at a typical cortical site in PZ. The first bar in the graph shows the mean and standard error (SE) of the rectified neuronal activity, measured during baseline resting just before the start of injection. The second bar in the graph shows the mean and SE of neuronal activity measured 10 min later, after the end of injection. As expected, the baseline neuronal activity was significantly reduced after the injection of muscimol ($t = -11.34$; $p < 0.0001$).

Figure 2B shows the effect of bicuculline injection (2.5 μg in 1 μl of saline at a rate of 0.1 μl/min) at another cortical site in PZ. As expected, the baseline neuronal activity was significantly increased by the injection of bicuculline ($t = 3.17$; $p = 0.003$). In addition to the increase in mean neuronal activity, the SE also increased markedly after the injection of bicuculline. This increase in both mean and variability was caused by a bursting behavior of the cells that is typically induced by bicuculline (e.g., Matsumura et al., 1991). By 15 min after the start of injection, these intense bursts of cell activity occurred at a variable rate of about one every 5–30 s.

Cell Bursts Induced by Bicuculline

The cortical site illustrated in Figure 2B was typical of sites in PZ in that the neurons responded to objects approaching or touching the contralateral side of the face (visual and tactile receptive fields shown in Figure 1D). In initial testing, electrical stimulation of this site evoked the expected set of defensive-like movements that appeared to protect the side of the face, including a squint and blink that was most pronounced on the contralateral side; a lifting of the contralateral upper lip; a shrugging of the contralateral shoulder; and a movement of the contralateral hand to a lateral position as if to block a threat. These movements matched our previous findings for electrical stimulation in PZ (Cooke and Graziano, 2004; Graziano et al., 2002a). One important question, therefore, was whether the same set of defensive-like movements would be evoked by the bursts of cell activity induced by bicuculline injection.

The black line in Figure 2C shows an example of a bicuculline-induced burst of neuronal activity. The trace shows the multineuron background activity, since the low impedance of the recording syringe did not easily allow for the isolation of single neurons. The red line shows the electromyographic (EMG) activity of the orbicularis muscle. The orbicularis surrounds the eye and participates in squinting and blinking, and its activity provides a measure of the defensive grimace. In this case, the EMG activity was measured from the side of the face contralateral to the injection site. The graph shows that, about 30 ms after the onset of the neuronal burst, a burst of activity occurred in the orbicularis muscle.

Figure 2D shows an average of 30 bicuculline-induced cell bursts. Each neuronal burst was defined as an increase in neuronal activity that exceeded the mean by four times the standard deviation. The neuronal bursts were aligned on the time point at which they rose above this threshold. The traces were rectified and averaged together. This mean shows a similar effect as in the single example shown in Figure 2C. On average, the burst in neuronal activity was followed by a burst in orbicularis muscle activity. The latency, or time between the onset of the neuronal burst and the onset of the EMG burst, was 30.13 ms (SE of 5.50). In a previous study using electrical stimulation of PZ to evoke activity in the orbicularis muscle, we obtained a mean latency of 31 ms ($SE = 1.89$) (Cooke and Graziano, 2003). Thus, in these respects the effect of chemical stimulation appears to be similar to the effect of electrical stimulation.

The orbicularis muscle is active during a defensive reaction, participating in blinking and squinting, including lowering of the eyebrow and raising of the skin under the eye. However, a defensive reaction can involve additional components (Strauss, 1929; Landis and Hunt, 1939; Schiff et al., 1962; King et al., 1992; Cooke and Graziano, 2003). Did these other components of a defensive reaction occur during the bicuculline-induced neuronal bursts? Figure 2E shows line drawings traced directly from video frames. These drawings illustrate the face between cell bursts and during a cell burst. The cell burst evoked a suite of movements including the following: a bilateral blink; a squinting of the musculature surrounding the eyes that was more pronounced on the side of the face contralateral to the injection; a lifting up of the lip, exposing the teeth; and a wrinkling upward of the skin on the snout that was more pronounced on the contralateral side. The ear is not visible from this video angle, though we did observe a folding backward of the ear against the head during each bicuculline-induced cell burst. The head remained fixed by the head bolt throughout the experiment, thus a turning aside of the head could not be observed. Figure 2F shows a view of the monkey's body, illustrating more of the movement

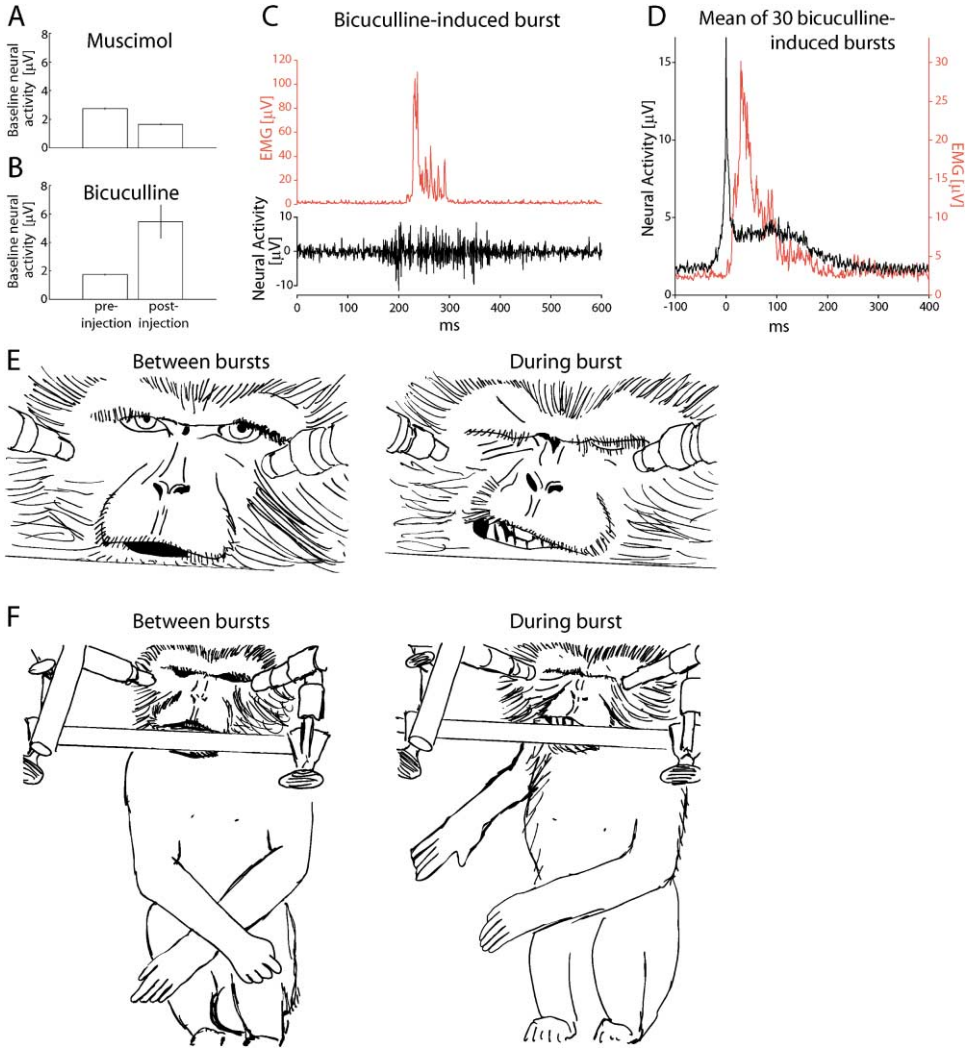

Figure 2. Effect of Bicuculline and Muscimol on Neuronal Activity

(A) Baseline neuronal activity at an example site in PZ before and after injection of muscimol. Multineuron activity was measured within a 1.5 s sample period just before injection and another 1.5 s sample period after injection. The data were divided into 100 ms bins. For each bin, the mean rectified neuronal activity was calculated. The mean and standard error (SE) across these bins is shown in the bar graphs. Muscimol caused a decrease in mean baseline.

(B) Baseline neuronal activity at an example site in PZ before and after injection of bicuculline. Bicuculline caused a mean increase in baseline.

(C) Example of a spontaneous burst of neuronal activity occurring after the injection of bicuculline. The black trace shows the multineuron activity, and the red trace shows the corresponding EMG activity from the contralateral orbicularis muscle, reflecting the facial grimace that followed the cell burst.

(D) Mean of 30 bicuculline-induced neuronal bursts. Multineuron activity was first rectified and integrated into 1 ms bins. Each burst was aligned on the time at which the neuronal activity exceeded 4 SD above the baseline. This time is plotted as zero on the x axis. On average, a burst of EMG activity in the orbicularis muscle followed the burst of cell activity in PZ.

(E) Line drawings of video frames showing the monkey's face between cell bursts and during a cell burst. During the burst, the monkey displayed the standard components of a defensive movement.

(F) Line drawings of video frames showing the monkey's body between cell bursts and during a cell burst.

during a cell burst. In the first picture, the monkey is in its normal resting posture with its hands crossed over its knees. In the second picture, in addition to the facial movements, the monkey also shrugged the contralateral shoulder and moved the arms toward the contralateral side. Measurements of the video images showed that during a cell burst the contralateral lip was elevated by a mean of 5.5 $\pm$ 1.6 mm and the contralateral hand moved by a mean of 173 $\pm$ 33 mm from an initial stationary position. These movements, evoked by chemically induced cell bursts in PZ, are standard components of a defensive reaction and do not resemble other categories of action such as putting food in the mouth, grasping, manipulating, or making a threat expression (which involves raising rather than lowering the brow, opening rather than closing the eyelids, and opening the jaw widely).

During qualitative testing, we found that multiple cell bursts in rapid succession and accompanying flinches could be triggered by stimuli approaching the monkey's

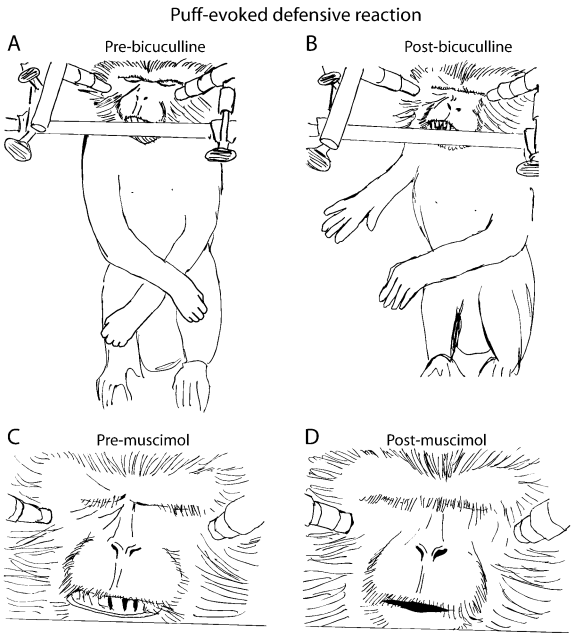

Figure 3. Effect of Bicuculline and Muscimol on Defensive Reactions to Air Puffs

(A) Air puff to the right (contralateral) cheek before injection of bicuculline into PZ.

(B) Air puff to the contralateral cheek after injection of bicuculline into PZ. The defensive reaction to the air puff is enhanced.

(C) Air puff to the contralateral cheek before injection of muscimol into PZ.

(D) Air puff to the contralateral cheek after injection of muscimol into PZ. The defensive reaction is reduced.

face, including slowly moving stimuli such as cotton swabs that previously caused little reaction from the monkey. This finding suggests that the neuronal disinhibition caused by bicuculline in PZ resulted in a behavioral disinhibition. Even normally subthreshold stimuli, such as the slowly moving cotton swab, became superthreshold and evoked a defensive reaction. We further tested this enhancement of the defensive reaction by using a stimulus that was normally superthreshold, that is, one that normally evoked a defensive reaction. The stimulus was an air puff delivered to the cheek. We tested whether injections of bicuculline into PZ enhanced the defensive reaction to this stimulus and whether injections of muscimol into PZ reduced the reaction. These tests are described in the following sections.

Effect of Chemical Injection on Air Puff-Evoked Movements: Examples

Figure 3A shows the effect of an air puff delivered to the monkey before injection of any drug into PZ. The camera angle was slightly off center. Although the two air nozzles appear to be asymmetrically placed in this line drawing of the video image, in fact they are arranged symmetrically to reach equivalent locations on the two cheeks. In this case, the puff was delivered to the right cheek. The figure shows a typical reaction of the monkey to the air puff. The facial movements include a blink, a squint, a lifting of the upper lip exposing the teeth, and an upward movement of the skin on the snout toward the eye, visible in the picture as a wrinkling of the skin on the right side of the nose. In addition, the shoulder on the side of the air puff became elevated. The blocking movement of the arm to a lateral position, a standard component of a strong defensive reaction, is typically present in the first few trials of a block of air puff trials and then habituates and becomes weak and intermittent on subsequent trials (Cooke and Graziano, 2003, 2004). On the trial depicted in this video frame, the arm on the side of the air puff lifted slightly from its resting position but did not move to a lateral blocking position. Thus, the reaction shown here is a moderate one. On average across these preinjection trials, air puff on the right cheek caused the right side of the lip to elevate by 2.4 $\pm$ 1.6 mm and caused the right hand to move 38 $\pm$ 13 mm from an initial stationary position.

Figure 3B shows the effect of an air puff to the right cheek tested 15 min later, after the injection of bicuculline into a site in PZ in the left hemisphere. The reaction to the air puff is now more pronounced. The facial grimace is of greater magnitude, including a tighter squint around the right eye, a greater lifting of the right side of the upper lip, and a greater degree of wrinkling of the skin on the right side of the snout. Also, a movement of the arms to a lateral, blocking position can be observed. Thus, the bicuculline injection appeared to enhance the monkey's defensive reaction to the air puff. On average across the postinjection trials, air puff on the right cheek caused the right side of the lip to elevate by 5.4 $\pm$ 2.5 mm. This lip elevation is significantly greater than that measured during the preinjection trials ($t = 4.82$; $p < 0.0001$). During the postinjection trials, the hand moved 142 $\pm$ 46 mm from an initial stationary position. This movement of the hand in reaction to the air puff was significantly greater than that measured during the preinjection trials ($t = 9.12$; $p < 0.0001$).

To study this enhancement more quantitatively, we measured the EMG activity in the orbicularis muscle. In a previous study examining defensive reactions to air puff in monkeys, we found that the activity of the orbicularis muscle, reflecting the facial squint and blink, is a particularly sensitive measure of the presence and magnitude of a defensive movement (Cooke and Graziano, 2003). This result is in agreement with previous studies indicating that the facial squint and blink is the most reliable part of the defensive reaction across many species (Strauss, 1929; Landis and Hunt, 1939; Schiff et al., 1962; King et al., 1992). Figure 4A shows the EMG activity data from the side of the face contralateral to the injection, during air puff to the contralateral cheek. The black trace shows a mean of 25 trials presented before bicuculline injection. This mean result follows a typical pattern including an intense, short-latency startle reflex followed by a lower-amplitude, more sustained second phase (Cooke and Graziano, 2003). The red trace shows a mean of 25 trials presented 15 min later, after bicuculline injection. Again, the reaction included an intense, short-latency startle reflex followed by a lower-amplitude, more sustained phase.

The baseline level of muscle activity, measured in the period before the onset of the air puff, was similar for pre- and postinjection tests, as can be seen by the

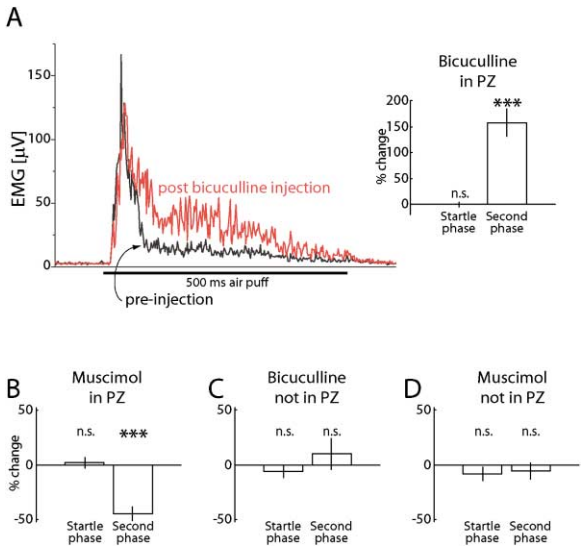

Figure 4. Examples of Muscimol and Bicuculline Injection inside and outside of PZ

(A) Bicuculline injection in PZ. Traces show EMG activity from orbicularis muscle contralateral to the injection, during air puff on the contralateral cheek, before (black trace) and after (red trace) bicuculline injection. Bar graph shows percent change (±SE) in EMG activity during the peak of the startle (36–50 ms after air puff onset) and during an equal time segment selected from the second phase of the response (200–214 ms after air puff onset). For this site, bicuculline caused a significant increase in the second phase of the response but not in the initial startle phase.

(B) Muscimol injection at another site in PZ caused a significant decrease in the second phase but not in the startle phase.

(C) Bicuculline injection outside PZ did not significantly affect either the startle phase or the second phase.

(D) Muscimol injection outside PZ did not significantly affect either the startle phase or the second phase.

similarity between the red and the black lines in Figure 4A. We calculated a percent change between preinjection and postinjection baseline, using a 500 ms time window prior to the start of the air puff. This percent change was not significantly different from zero (percent difference = 3.6%; SE = 9.77; t = 0.37; p = 0.71). Thus, bicuculline injected in PZ did not change the tonic level of activity in the muscle.

During this baseline period between air puffs, the video record indicated that the monkey made frequent lid and brow movements, such as those that accompanied gaze shifts, facial expressions, and spontaneous blinks. The lack of effect in this baseline period suggests that these ongoing, nondefensive movements were not altered by the bicuculline injection. To address this question more directly, we used the video record to identify the times during the intertrial interval when the monkey was performing a facial action such as brow movement or spontaneous blinking. We analyzed the orbicularis EMG activity during these movement times. The orbicularis activity rose significantly during these movements, indicating that the muscle did participate in the movements (EMG activity during movement was elevated above baseline by 45.8%; SE = 9.3; t = 4.90; $p < 0.0001$). However, this EMG activity during the nondefensive movements in the intertrial interval was not significantly affected by bicuculline. The percent change from preinjection to postinjection tests was not significantly different from zero (percent change = 10.1%; SE = 16.17; t = 0.62; p = 0.54).

The startle phase of the defensive reaction was also unaffected by bicuculline, rising to a similar peak for both pre- and postinjection tests, as can be seen in Figure 4A. The percent change in this startle reaction between the pre- and postinjection tests was not significantly different from zero (percent change = −0.52%; SE = 5.13; t = −0.101; p = 0.994).

The sustained phase of the response, however, was elevated after bicuculline injection. This elevation in EMG activity caused by bicuculline remained through the duration of the air puff. The percent change between the pre- and postinjection tests was significantly greater than zero (percent change = 157%; SE = 26.66; t = 5.899; $p < 0.0001$).

When the air puff ended, the effect of bicuculline disappeared, as indicated by the convergence of the pre- and postinjection traces. For the time period just after the puff ended (100–500 ms after puff offset), the percent change between pre- and postinjection tests was once again not significantly different from zero (percent change = 12.23%; SE = 6.63; t = 1.84; p = 0.07).

These results indicate that bicuculline injected into PZ enhanced the activity of the orbicularis muscle, but in a highly specific manner, during the second, sustained phase of the defensive reaction to the air puff, not during baseline resting, during the startle reflex, or during nondefensive movements made in the intertrial interval.

Figures 3C and 3D show line drawings of video frames corresponding to a muscimol injection into PZ. Figure 3C shows the effect of an air puff delivered to the right cheek of the monkey before the injection of any drug into PZ. Figure 3D shows the effect of air puff tested 15 min later, after the injection of muscimol into a site in PZ. The reaction to the air puff is visibly reduced after muscimol injection. The eyelids are closed, but the facial grimace is no longer apparent. On average across preinjection trials, air puff on the right cheek caused the right side of the lip to elevate by 2.5 ± 1.4 mm, whereas on postinjection trials, the lip elevated by 1.5 ± 1.3 mm. This reduction in extent of lip elevation was significant (t = 2.40; p = 0.021). The hand movement was not measured, because in this test, to better capture subtle facial movements after muscimol injection, the camera's field of view was limited to the face only.

Figure 4B shows the results from the orbicularis muscle for this site. The muscimol injection caused a reduction in the orbicularis activity, reflecting a reduced facial squint and blink. This reduction, however, was not apparent in the initial startle phase of the monkey's reaction to the air puff (t = 0.364; p = 0.921) but was statistically significant in the second phase of the response (t = −6.784; $p < 0.0001$). Note that the effects of bicuculline (Figure 4A) and muscimol (Figure 4B) were opposite, one increasing the second phase of the defensive reaction and the other decreasing it. These effects, therefore, cannot be explained by pressure from the injection, presence of the cannulus, or other nonspecific factors.

Figures 4C and 4D show the results of injecting bicuculline and muscimol at cortical sites outside of PZ. These sites were located in the precentral gyrus within

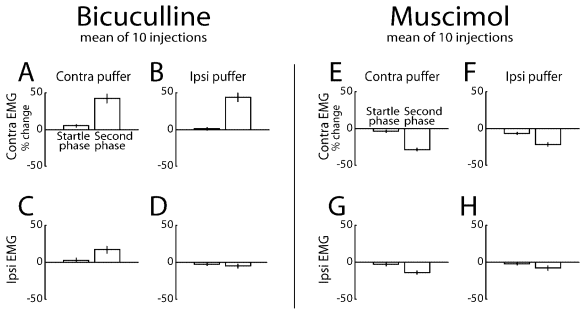

Figure 5. Mean Result from Ten Bicuculline Injections and Ten Muscimol Injections in PZ

(A–D) In conditions (A)–(D), bicuculline injection caused an overall increase in the second phase of the response. This second phase was further tested with a 2 × 2 ANOVA (EMG activity contralateral or ipsilateral to injection × air puff on contralateral or ipsilateral cheek). Main effect of EMG location was significant ($F = 46.445$, $p < 0.0001$); main effect of puff location was not significant ($F = 3.482$, $p = 0.062$); interaction was significant ($F = 4.969$, $p = 0.026$). (E–H) In conditions (E)–(H), muscimol injection caused a decrease in the second phase of the response. This second phase was further tested with a 2 × 2 ANOVA indicating a significant main effect of EMG location ($F = 20.796$, $p < 0.0001$) and a significant main effect of puff location ($F = 4.137$, $p = 0.042$) but no significant interaction ($F = 0.033$, $p = 0.855$).

2 mm of PZ. For both chemicals, neither the startle nor the second phase of the defensive response was significantly affected. (Bicuculline injection: startle phase, $t = -1.052$, $p = 0.515$; second phase, $t = 0.659$, $p = 0.766$. Muscimol injection: startle phase, $t = -1.333$, $p = 0.352$; second phase, $t = -0.723$, $p = 0.726$).

Effect of Chemical Injection on Air Puff-Evoked Movements: Group Data

We tested 20 injection sites in PZ: ten with bicuculline (four in monkey 1 and six in monkey 2) and ten with muscimol (six in monkey 1 and four in monkey 2). The reason for the relatively small number of sites is that the pressure injections can cause cumulative damage to the cortex, especially in a relatively small cortical region such as in the present experiment (2–3 mm in diameter; see the Experimental Procedures for details of locating PZ).

Figure 5A shows the mean result for bicuculline, for the condition in which the puff was presented to the contralateral cheek and the EMG activity was measured in the contralateral orbicularis muscle. Bicuculline had a small, nonsignificant effect on the startle phase of the defensive response ($t = 2.13$; $p = 0.066$) and caused a pronounced, significant enhancement of the second phase of the response ($t = 6.39$; $p < 0.0001$). This mean result across ten injection sites is similar to the result for the example site shown in Figure 4A.

Figure 5E shows the mean result for muscimol, for the condition in which the puff was presented to the contralateral cheek and the EMG activity was measured in the contralateral orbicularis muscle. Muscimol had a small, nonsignificant effect on the startle phase of the defensive response ($t = -2.056$; $p = 0.079$) and caused a pronounced, significant reduction of the second phase of the response ($t = -11.41$; $p < 0.0001$). This mean result across ten injection sites is similar to the result for the example site shown in Figure 4B.

These results show that chemical manipulation of sites in PZ affects the second phase of the monkey's defensive reaction to an air puff. But what is the nature of the effect? Did the chemical manipulation alter the strength of the motor output, or did it alter the perceived salience of the air puff stimulus? Electrical stimulation of PZ evokes a short-latency movement that is largest on the contralateral side of the body (Cooke and Graziano, 2004). Thus, one hypothesis is that chemical manipulation of PZ should cause a motor effect, increasing or decreasing the defensive movements in the musculature on the contralateral side. However, PZ neurons respond to sensory stimuli on the contralateral side of the body (Rizzolatti et al., 1981; Gentilucci et al., 1988; Fogassi et al., 1996; Graziano et al., 1997, 1999; Graziano and Gandhi, 2000); thus, another hypothesis is that chemical manipulation of PZ should cause a sensory enhancement or sensory neglect, altering the processing of stimuli located on the contralateral side and thereby affecting the defensive reaction to those stimuli. To test these motor and sensory possibilities, we used a 2 × 2 design, measuring muscle activity on the left or right side of the face during air puff delivered to the left or right cheek.

As shown in Figures 5A–5D, bicuculline increased the second phase of the defensive response. This increase was significantly greater for the contralateral muscle (Figures 5A and 5B) than for the ipsilateral muscle (Figures 5C and 5D) (analysis of variance [ANOVA], main effect of muscle location, $F = 46.445$, $p < 0.0001$). This result is consistent with the enhancement of a motor output that projects primarily to the contralateral side.

The data show a smaller effect of stimulus location. The effect of bicuculline was on average larger when the puff was presented on the contralateral side (Figures 5A and 5C) than when the puff was presented on the ipsilateral side (Figures 5B and 5D). This effect of stimulus location approached but did not reach significance (main effect of stimulus location, $F = 3.482$, $p = 0.062$). The effect of stimulus location can be seen more clearly by comparing Figures 5C and 5D. Here, the effect of bicuculline was apparent for stimuli presented to the contralateral cheek (Figure 5C) and absent for stimuli presented to the ipsilateral cheek (Figure 5D). This difference resulted in a significant interaction term in the ANOVA (interaction, $F = 4.969$, $p = 0.026$).

The results of this analysis indicate that the effect of bicuculline was primarily motor specific, affecting the muscle output on the contralateral side of the face more than that on the ipsilateral side. To a lesser extent, it also showed some sensory specificity, affecting reactions to stimuli presented on the contralateral side of the face more than on the ipsilateral side.

As shown in Figures 5E–5H, muscimol caused a reduction in the second phase of the defensive response. The reduction in defensive reaction was significantly more pronounced for the contralateral muscle (Figures 5E and 5F) than for the ipsilateral muscle (Figures 5G and 5H) (main effect of muscle location, $F = 20.796$, $p < 0.0001$). This result is consistent with the reduction of a motor output that projects primarily to the contralateral side.

The data show a smaller effect of stimulus location:

the reduction in defensive reaction was significantly more pronounced when the air puff was presented to the contralateral side of the face (Figures 5E and 5G) than when the air puff was presented to the ipsilateral side of the face (Figures 5F and 5H). This effect of stimulus location reached significance (main effect of stimulus location, $F = 4.137$, $p = 0.042$). The interaction between the stimulus location and the muscle location was not significant (interaction, $F = 0.033$, $p = 0.855$).

Thus, just as for bicuculline, the effect of muscimol was primarily motor specific, affecting the muscle output on one side of the face more than that on the other side. To a lesser extent, it also showed some sensory specificity, affecting reactions to stimuli presented on one side of the face more than on the other side.

Discussion

In this experiment, we manipulated the level of γ-aminobutyric acid (GABA) inhibition in PZ, a specific region of the precentral gyrus, and measured the monkey's defensive reactions to an air puff. Increasing inhibition by injection of the GABA agonist muscimol caused a decrease in the magnitude of the monkey's defensive reaction. Decreasing inhibition by injection of the GABA antagonist bicuculline caused an increase in the magnitude of the monkey's defensive reaction, as well as apparent spontaneous defensive actions.

We measured muscle activity on the left and right side of the face during air puff presented to the left or right cheek. In this fashion, we were able to test whether the location of the motor output or the location of the sensory input was better able to account for the effect of the drug. The pattern of results suggested that chemical manipulation of PZ mainly affected the motor output to the contralateral cheek. Superimposed on this motor effect was also a smaller sensory effect, in which the chemical injection altered the monkey's reactions to stimuli presented to the contralateral side of the face. In this sense, inactivation of PZ caused a partial contralateral sensory neglect, and activation caused a contralateral sensory enhancement. Contralateral sensory neglect on damage to the precentral gyrus has been reported before (Husain and Kennard, 1996; Rizzolatti et al., 1983). However, the contralateral sensory neglect and enhancement obtained in the present experiment was smaller in magnitude than the contralateral motor reduction and enhancement.

The contralateral motor effect did not take the form of a general depression or excitation of the muscles. It was not apparent in the baseline muscle activity. It was not apparent during nondefensive movements made during the intertrial interval. It was also not apparent during the startle reflex. It was obtained specifically in the second, post-startle phase of the defensive reaction. It is this phase of a defensive reaction in which a spatially directed movement is made to retract from or to block a potentially dangerous stimulus (Strauss, 1929; Landis and Hunt, 1939; Schiff et al., 1962; King et al., 1992; Cooke and Graziano, 2003). These results are therefore consistent with the hypothesis that PZ participates specifically in the spatially directed, second phase of a defensive reaction.

The present results are consistent with the known physiological properties of neurons in PZ. Neurons in PZ respond to sensory stimuli near and approaching the body and have sensory receptive fields that are typically on the contralateral side of the body (Rizzolatti et al., 1981; Gentilucci et al., 1988; Fogassi et al., 1996; Graziano et al., 1997, 1999; Graziano and Gandhi, 2000). Electrical stimulation of PZ evokes short-latency movements that resemble defensive reactions, as if the monkey were protecting the part of the body covered by the sensory receptive fields of the stimulated neurons (Graziano et al., 2002a; Cooke and Graziano, 2004). The present results show that chemical intervention in PZ can affect the sensorimotor process of defending the body surface from noxious stimuli.

An alternative hypothesis is that PZ, with its visual receptive fields confined to the space near the body, serves a general function related to interaction with objects near the body, including reaching, grasping, and especially bringing the hand toward the face. This hypothesis seems unlikely, since stimulation of PZ, whether electrical or chemical, does not evoke reaching, grasping, or bringing of the hand to the face but rather evokes movements that closely resemble defensive reactions (Cooke and Graziano, 2003). Enhancement of PZ activity with bicuculline did not cause the monkey to spontaneously reach toward nearby objects or to spontaneously put the hand to the mouth but rather caused spontaneous apparent flinches. These results suggest that defense of the body surface is at least a dominant function of PZ. Electrical stimulation in nearby regions of cortex, outside of PZ, can evoke a range of different movements. For example, feeding-like movements, including a movement of the hand toward the mouth, a grip movement of the hand, and an opening of the mouth, can be evoked from a region of cortex that is ventral and sometimes also anterior to PZ (Graziano et al., 2002a).

A Possible Cortical Pathway for the Defense of the Body Surface

A basic function of the motor system of all animals is to protect the body from attack or collision (Hediger, 1955; Schiff, 1965; Dosey and Meisels, 1969). Areas in the pigeon brain, locust brain, and fly brain have been implicated in the detection of looming visual stimuli and the control of avoidance (Sun and Frost, 1998; Rind, 2002; Schuster et al., 2002; Tammero and Dickinson, 2002). Portions of the rat superior colliculus and other subcortical structures may participate in avoidance behaviors (Dean et al., 1989; Brandao et al., 1993). Several subcortical nuclei have been linked to the startle reflex (Koch, 1999; Yeomans et al., 2002). We suggest that defensive behavior in primates, in particular the complex, spatially directed, second phase of a defensive reaction, may be partly cortically mediated by area PZ.

Other cortical areas may also participate in defensive reactions. For example, neurons in the ventral intraparietal area (VIP) respond to stimuli near, approaching, and touching the face (Colby et al., 1993; Schaafsma and Duysens, 1996; Duhamel et al., 1998; Bremmer et al., 2002); electrical stimulation of VIP evokes defensive-like movements (Thier and Andersen, 1998; Cooke et al., 2003); and VIP and PZ are monosynaptically connected

(Luppino et al., 1999; Lewis and Van Essen, 2000). One hypothesis, therefore, is that VIP and PZ participate in the same sensorimotor pathway. In this hypothesis, VIP is closer to the sensory end, with a relative emphasis on the representation of objects moving near the body, whereas PZ is closer to the motor end, with a relative emphasis on the coordination of appropriate movements. It will be important to chemically activate and inactivate VIP to determine if defensive movement can be altered and if the effects are primarily sensory or motor.

In the visual system, one organizing principle appears to be an ethological one. The processing of faces and other ethologically relevant stimuli appear to have cortical hot spots; each hot spot processes a range of stimuli but is relatively specialized for one stimulus class (Kanwisher et al., 1997; Haxby et al., 2001). PZ may provide an example in the motor system of a relative hot spot for the ethologically important function of defense of the body surface, although PZ may of course have other sensory and motor functions not tested in the present experiment.

Experimental Procedures

All husbandry, surgical, and behavioral procedures were approved by the Princeton University Institutional Animal Care and Use Committee and the attendant veterinarian and were in accordance with NIH and USDA guidelines. For details, see Cooke and Graziano (2004). Two adult male *M. fascicularis*, each with a recording chamber embedded in an acrylic skullcap over the precentral gyrus, were trained to sit quietly with their heads fixed but their limbs free to move. During the daily recording sessions, a Crist recording microsyringe (Hagerstown, MD) mounted on a microdrive (Narishige, Tokyo) was lowered into the precentral gyrus while neuronal activity was monitored over a loudspeaker. Somatosensory responsiveness was studied using manual palpation, manipulation of joints, gentle pressure, and stroking with cotton swabs. Visual responsiveness was studied using handheld visual stimuli. Most multimodal neurons in PZ do not respond to visual stimuli projected onto a tangent screen, even when the screen is placed close to the face (Graziano et al., 1997). Instead, they respond best to objects near the animal. Therefore, we used real objects, such as a ping-pong ball mounted on the end of a rod, to study visual responses.

Electrical Microstimulation

Each site was also tested with electrical stimulation using an S88 stimulator and two SIU6 stimulus isolation units (Grass, West Warwick, RI). Stimulation consisted of a 500 ms train of pulses presented at 200 Hz. Each pulse had a negative followed by a positive phase, each phase 0.2 ms in duration. Current was set between 25 and 200 μA and was measured via the voltage drop across a 1 KΩ resistor in series with the return lead of the stimulus isolation units. For details on stimulation procedures in PZ, see Cooke and Graziano (2004).

Air Puff Stimulus

To evoke defensive movements, an air nozzle directed a 0.5 s stream of air at the monkey's skin from a distance of 5 cm. Pressures were typically set between 5 and 30 pounds per square inch (PSI). For most experiments, the pressure was set to 15 PSI. Two nozzles were used, one directed at each cheek. The two nozzles were actuated in a pseudorandom schedule with an interpuff interval of 15 s. In pilot tests, this interval was found to result in minimal habituation. A block of 50 trials (25 puffs on each cheek) was presented before injection of chemical agent into the cortex, and a second block of 50 trials was presented after injection.

EMG Recordings

During air puff trials, EMG activity was measured bilaterally in the orbicularis muscle. Fine insulated stainless steel wires were threaded into a 22G syringe needle and inserted into the muscle. The wires had an exposed tip of 1–2 mm. Three wires spaced about 5 mm apart were inserted in each muscle to provide input to a differential amplifier and its ground (single neuron amplifier model 1800; A-M Systems, Sequim, WA). The amplifier filters were set with a low cutoff at 300 Hz and a high cutoff at 1000 Hz. The signal was digitally sampled every 2 ms and rectified.

Injection of Bicuculline and Muscimol

Each day, the Crist recording microsyringe was used to characterize the neuronal responses and the effect of electrical stimulation at a cortical site. Then, the microsyringe was used to inject 1–2 μl of muscimol (10 μg/μl in saline) or 1 μl of bicuculline methiodide (2.5–5 μg/μl in saline) at 0.1–0.2 μl/min (Hikosaka and Wurtz, 1985; Matsumura et al., 1991; Dias and Segraves, 1999; Malpeli, 1999; Fogassi et al., 2001). To minimize the possibility of leakage of the drug before the injection, a 0.5–1.0 μl plug of saline was withdrawn into the tip of the cannulus. The saline plug was injected first, followed by the drug. The effects of the drug were verified by recording multineuron activity during the injection. Muscimol eliminated neuronal spiking, whereas bicuculline caused periodic, spontaneous bursts of activity. Because of the possible tissue damage caused by repeated pressure injections, and because of the small size of area PZ (2–3 mm diameter), it was not possible to test a large number of injections. We tested ten muscimol injections (six in monkey 1, four in monkey 2) and ten bicuculline injections (four in monkey 1, six in monkey 2) in PZ.

Data Analysis

For each site, we quantified the effect of the injection by calculating the percent change in the air puff-evoked EMG activity between the preinjection and the postinjection test. This percent change was calculated for the initial startle phase of the EMG response and also for the second, more sustained phase of the response. One possible concern in comparing the startle phase to the second phase is that the startle peak is typically brief (ms timescale), and thus the analysis can only be done on a limited time window, whereas the second phase of the response continues until the end of the air puff, and thus the analysis can be done on a longer time window. If the full duration of the second phase were compared to the startle phase, the smaller amount of data in the startle phase might result in a lower likelihood of detecting a reliable effect, thus falsely supporting the hypothesis that the chemical injection affects mainly the second phase. To ensure that the statistical tests were not biased in this fashion, we selected a sample of data from the peak of the startle phase and an equally brief sample from the second phase. The analysis window for the startle phase was from 36 to 50 ms after air puff onset. (This analysis window encompassed the average time of the peak of the startle.) The analysis window for the second phase of the response was from 200 to 214 ms after air puff onset (an arbitrary, representative sample from the second phase). We tried analyzing samples at different times during the second phase and also tried using a longer time window to encompass more of the second phase and obtained similar results.

Identification of PZ

PZ was identified on the basis of a convergence of three criteria. (1) Neurons in PZ responded to tactile stimuli on the face, arms, or torso and to nearby visual stimuli, whereas surrounding cortical areas did not have multimodal responses. (2) Electrical stimulation of PZ evoked defensive-like movements, whereas stimulation of surrounding cortical areas evoked other types of movement (Cooke and Graziano, 2003, 2004; Graziano et al., 2002a). (3) PZ was located on the cortical surface just posterior to the bend in the arcuate sulcus. The sulcal pattern was inferred by monitoring the pattern of cellular activity and silence as the electrode was advanced and by obtaining electrically evoked saccadic eye movements in the frontal eye fields just anterior to the sulcus. Both monkeys are still in use in experiments.

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