Identification and Functional Characterization of Brainstem Cannabinoid CB_2 Receptors

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The presence and function of CB_2 receptors in central nervous system (CNS) neurons are controversial. We report the expression of CB_2 receptor messenger RNA and protein localization on brainstem neurons. These functional CB_2 receptors in the brainstem were activated by a CB_2 receptor agonist, 2-arachidonoylglycerol, and by elevated endogenous levels of endocannabinoids, which also act at CB_1 receptors. CB_2 receptors represent an alternative site of action of endocannabinoids that opens the possibility of nonpsychotropic therapeutic interventions using enhanced endocannabinoid levels in localized brain areas.

Endocannabinoids, anandamide, and 2-arachidonoylglycerol (2-AG) are lipid mediators that act at CB_1 and CB_2 receptors (*1*, *2*). Their actions are terminated through cellular uptake facilitated by a putative endocannabinoid transporter, followed by intracellular enzymatic hydrolysis. Two degradative enzymes for endocannabinoid metabolism are known; fatty acid amide hydrolase (FAAH) preferentially degrades anandamide, and monoacylglycerol lipase preferentially degrades 2-AG (*1*, *3*, *4*). The CB_1 receptor is highly expressed in the CNS, where cannabinoids act at presynaptic CB_1 receptors to elicit changes in the synaptic efficacy of neuronal circuits (*5*). The CB_2 receptor has been found outside the CNS and is particularly associated with immune tissues, such as the spleen and thymus, as well as in various circulating immune cell populations (*6*). In the CNS, CB_2 receptor mRNA has been reported in cerebellar granule cells (*7*), and CB_2 receptors have been described on perivascular microglial cells and in cultured cerebrovascular endothelium (*8*, *9*). CB_2 receptor expression is enhanced on glia in neuritic plaques and on immune cells in simian immunodeficiency virus encephalitis (*10*, *11*). To date, however, the CB_2 receptor protein has not been localized on central neurons, and the effects of endocannabinoids in the brain have always been attributed to an action at CB_1 receptors.

We found CB_2 receptor mRNA expression in the brain (cerebellum, cortex, and brainstem) and spleen of the rat using reverse transcription polymerase chain reaction (RT-PCR) (Fig. 1). Sequencing of the 472–base pair PCR product showed that the products amplified from the spleen, cortex, and brainstem were identical to the rat CB_2 receptor sequence (*12*, *13*). Using quantitative real-time RT-PCR, we determined that the brainstem contained $1.5 \pm 0.9\%$ of the CB_2 receptor mRNA found in the spleen (fig. S1) (*14*). Amplification of CB_2 receptor from the brainstem was not due to genomic contamination of our sample, because amplification of RNA that was not reverse-transcribed did not lead to the generation of a product. Furthermore, our real-time PCR primers spanned intron-exon borders, which ensured that the only product that could be amplified was the spliced mRNA.

We next investigated whether CB_2 receptor protein could be detected by Western blotting and/or immunohistochemistry (*14*). Western blotting for the CB_2 receptor in the brainstem and cerebellum revealed three bands at about 45, 55, and 60 kD (Fig. 1), similar to previous reports in spleen (*15*). In the brainstem, CB_2 receptor immunoreactivity was found in neurons within the dorsal motor nucleus of the vagus (DMNX), the nucleus ambiguous, and the spinal trigeminal nucleus; glial cells and blood vessels did not express detectable CB_2 receptor immunoreactivity (*16*). Preincubation with the cognate peptide of the CB_2 receptor antibody completely abolished cellular staining. These results are in contrast to the conclusions drawn by Derbenev *et al.* (*17*), who reported no CB_2 mRNA or receptor protein in similar regions of the rat brainstem. However, evaluation of their figures reveals a faint band of immunoreactivity in Western blots, consistent with our observations, and their RT-PCR primers were directed against different regions of the CB_2 receptor mRNA. The differences in the conclusions drawn likely reflect the low abundance of CB_2 receptor in the brain relative to the spleen and the choice of RT-PCR primers.

We next pursued the functional significance of this observation. The major psychoactive cannabinoid, Δ^9-tetrahydrocannabinol (THC), is effective in the treatment of nausea and vomiting (emesis) (*18*). THC acts on neurons in the dorsal vagal complex of the brainstem, the site of integration of emetic reflexes that includes the nucleus of the solitary tract (NTS), the area postrema, and DMNX (*18*, *19*), where we found CB_2 receptor expression. These well-characterized actions of CBs have been demonstrated in the ferret. Because of the inability of rodents to vomit, we verified our observations of the receptor distribution in this species (Fig. 1). Indeed, as in the rat, we observed major bands of immunoreactivity in Western blots at about the same relative molecular weights and a similar distribution of CB_2 receptor expression in neurons of the DMNX.

These results led us to investigate whether endocannabinoids could act at the CB_2 receptor in the brainstem to inhibit emesis. Using morphine-6-glucuronide (M6G) as an emetic stimulus, we found that both anandamide and 2-AG dose-dependently reduced emesis in the ferret (Fig. 2) (*14*). Using selective CB receptor antagonists, we attempted to reverse the actions of these endocannabinoids. The antiemetic effect of anandamide was almost completely reversed by the selective CB_1 receptor antagonist AM251 but was not significantly altered by the CB_2 antagonist AM630, which is consistent with the fact that the anandamide is not very efficacious at CB_2 receptors (*2*) and indicates that the dose of AM630 used does not antagonize CB_1 receptors. In contrast, the antiemetic effects of 2-AG were reversed by both AM251 and AM630 (Fig. 2). Thus, the CB_2 receptor may be functionally expressed in the ferret brainstem and could be targeted by an endocannabinoid. As we observed the ferrets during our studies, we noted that 2-AG administration was far less sedating than anandamide (Fig. 2). This action is consistent with a preferential effect at a CB_2 receptor, because CB_1 receptor activation *in vivo* is associated with sedation (*2*).

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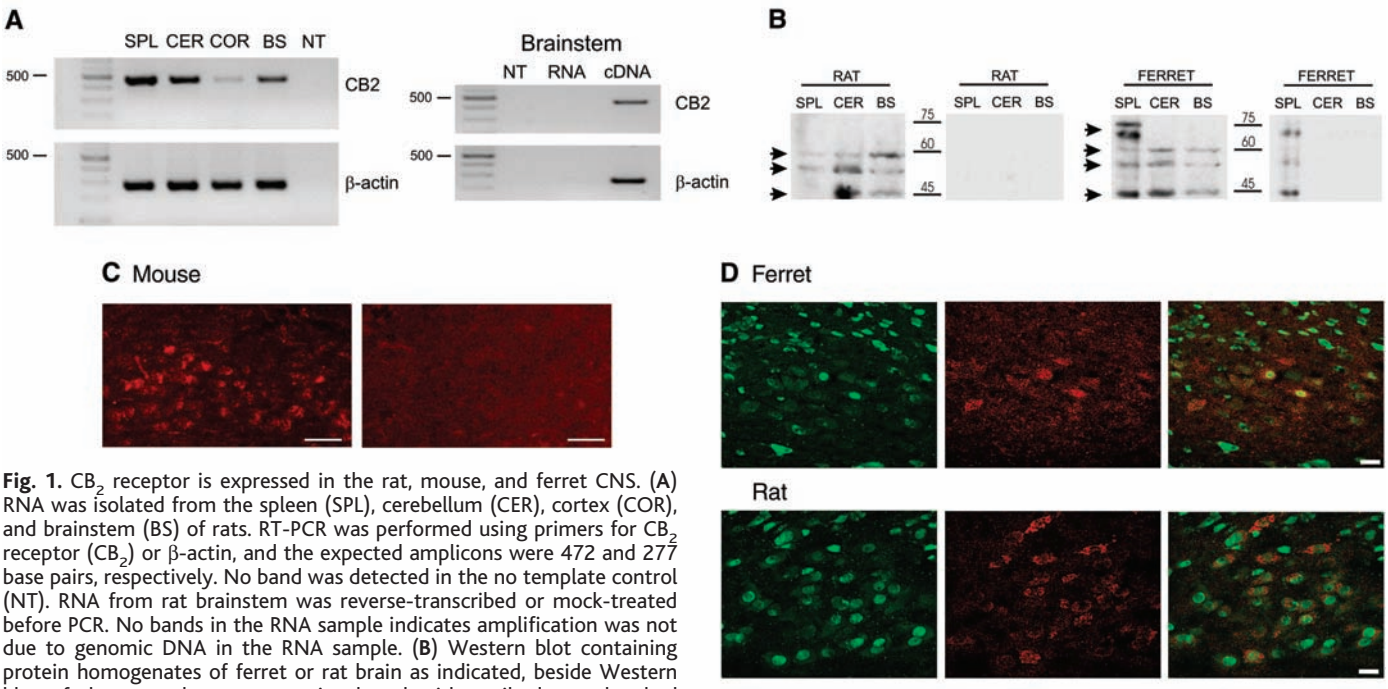

Fig. 1. CB_2 receptor is expressed in the rat, mouse, and ferret CNS. (**A**) RNA was isolated from the spleen (SPL), cerebellum (CER), cortex (COR), and brainstem (BS) of rats. RT-PCR was performed using primers for CB_2 receptor (CB_2) or β-actin, and the expected amplicons were 472 and 277 base pairs, respectively. No band was detected in the no template control (NT). RNA from rat brainstem was reverse-transcribed or mock-treated before PCR. No bands in the RNA sample indicates amplification was not due to genomic DNA in the RNA sample. (**B**) Western blot containing protein homogenates of ferret or rat brain as indicated, beside Western blot of the same homogenates incubated with antibody preabsorbed with control peptide. Major bands were observed at about 45, 55, and 60 kD in rat and ferret brainstem (arrows; $n = 6$). In the ferret spleen, higher molecular weight bands were also observed (arrow). In the rat brain and spleen and ferret brain, we completely preabsorbed all the immunoreactive bands. However, in the ferret spleen, bands were substantially reduced, but not completely preabsorbed, with the concentration of peptide used. (**C**) CB_2 receptor immunoreactivity in the dorsal motor nucleus of the vagus of wild-type (left) and $CB_2^{-/-}$ (right) mice (*30*). Note the lack of immunoreactive cell bodies in the knockout mouse ($n = 3$ per group). Scale bar, 50 μm. (**D**) Immunoreactivity for the neuronal nuclear marker NeuN (left, green) and CB_2 receptor immunoreactivity (center, red) in neurons of the dorsal motor nucleus of the vagus of the ferret and rat, as indicated. Overlay (right) of NeuN and CB_2 receptor illustrates that CB_2 receptor immunoreactivity is present in neurons of both the ferret and rat ($n = 4$), where it is localized on the cell membrane and in the cytoplasm of the neurons. Scale bars, 20 μm.

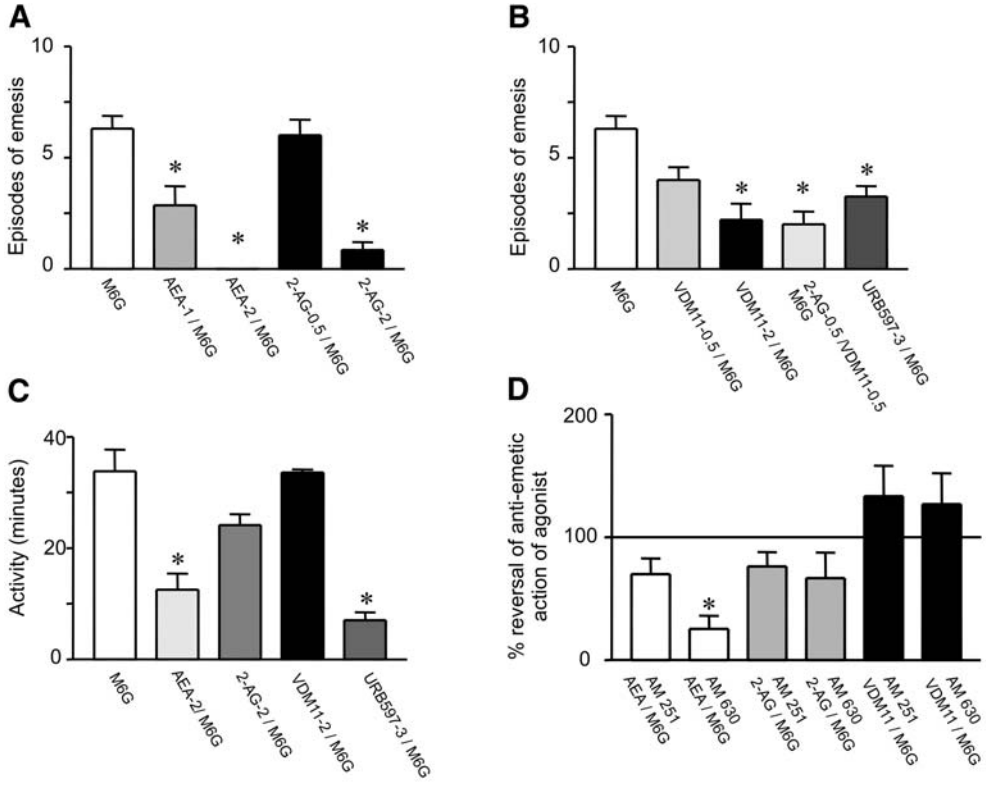

Fig. 2. Endocannabinoids reduce emesis induced by M6G. (**A**) Episodes of emesis after treatments of emetic agent alone, M6G (0.05 mg/kg, subcutaneously; $n = 10$) or the following treatments ($n = 3$ to 7) preceding M6G; anandamide (AEA-1, 1 mg/kg; and AEA-2, 2 mg/kg); and 2-arachidonoyl glycerol (2-AG-0.5, 0.5 mg/kg; and 2-AG-2, 2 mg/kg). AEA and 2-AG had no emetic actions when given alone. (**B**) Episodes of emesis after M6G alone; or with the transport inhibitor (VDM11-0.5, 0.5 mg/kg; and VDM11-2, 2 mg/kg); paired ineffective doses of VDM11 and 2-AG (0.5 mg/kg); an FAAH inhibitor URB 597 (3 mg/kg). (**C**) Activity during the 60-min observation after the treatments indicated. (**D**) CB_1 antagonist AM251 (5 mg/kg) reversed the effects of anandamide (AEA, 2 mg/kg), 2-AG (2 mg/kg), and VDM11 (2 mg/kg). In contrast, the CB_2 antagonist AM630 (5 mg/kg) did not reverse the effect of AEA (2 mg/kg), but effectively reversed the action of 2-AG (2 mg/kg) and VDM11 (2 mg/kg). In the presence of AM630 (5 mg/kg), there were 3.4 ± 0.7 emetic episodes ($n = 5$) in animals given a lower dose of AEA (1 mg/kg), which is not significantly different from the 2.8 ± 1.3 emetic episodes ($n = 5$) in the absence of AM630. AM251 and AM630 had no emetic actions when given alone. AM630 did not potentiate the effects of M6G, whereas AM251 enhanced emesis as previously described (*17*). Results are expressed as percentage of number of episodes of emesis induced by M6G. *Significant differences compared with M6G alone, $P < 0.05$. Bars represent mean ± SEM.

Although these data were strongly indicative of the actions of 2-AG at CB_2 receptors, it was important to evaluate whether endogenously released endocannabinoids reduce emesis through an action at CB_2 receptors. We used two approaches that would raise endocannabinoid levels in the brain. First, we tested whether the selective endocannabinoid reuptake inhibitor VDM11 (*20*) inhibited emesis. Second, we tested whether an FAAH inhibitor (URB 597) (*21*) reduced emesis. In both cases, we blocked or reduced the extent of emesis induced by M6G (Fig. 2). We extended these studies to find whether VDM11 would potentiate the effects of 2-AG. We used doses of VDM11 and 2-AG that alone did not significantly reduce emesis. When VDM11 was given together with 2-AG, we found a significant attenuation in the number of emetic episodes (Fig. 2). URB 597 strongly sedated animals, which suggested that this compound may selectively enhance anandamide levels. Conversely, VDM11 had no sedative effects, and the antiemetic effects were reversed by both AM251 and AM630, which suggested that VDM11 may preferentially affect 2-AG levels.

If the arguments made above were correct and if endocannabinoids undergo increased turnover in response to an emetic stimulus, treatment with VDM11 or URB 597 would be expected to increase the levels of endocannabinoids in the brainstem. We investigated this using a model of emesis under anesthetized conditions, so that the brains could be rapidly removed and frozen in order to limit the inherent instability of endocannabinoids in tissue samples (*14*). Ferrets were given hypertonic saline as an emetic stimulus because M6G is an inconsistent emetic in anesthetized animals. Levels of anandamide and total arachidonoyl glycerol [which reflect tissue 2-AG levels (*14*)] were measured in the brainstem and, for comparison, the cerebellum. Levels of endocannabinoids in the ferret under basal conditions or after emesis were comparable to levels previously found in rodents (Fig. 3). As expected from the pharmacological experiments described above, VDM11 specifically increased levels of total arachidonoyl glycerol in the brainstem and cerebellum, whereas pretreatment with URB 597 only increased the levels of anandamide in the brainstem.

These results led us to investigate whether selective CB_2 receptor agonists reduced emesis. We observed no statistically significant reductions in emesis in animals given the CB_2 receptor agonists AM1241 (1 or 2 mg/kg) or JWH 133 (1 or 5 mg/kg) before M6G (*22*). This finding was not completely surprising, as inhibitors of endocannabinoid inactivation can be more efficacious than "direct" agonists (*23*–*25*). Furthermore, these data suggest that CB_2 receptor activation alone is not sufficient to inhibit emesis and that, under appropriate conditions, for example, those produced by inhibiting endocannabinoid inactivation, the CB_2 receptor can be activated in local brain regions together with CB_1 receptors and can inhibit emesis. This hypothesis was supported by a significant reduction in episodes of emesis (7.1 ± 0.5 to 5.0 ± 0.7; $n = 6$ to 10; $P < 0.05$) when anandamide (0.5 mg/kg) and AM1241 (1 mg/kg) were administrated together at doses that were not antiemetic when either compound was given alone with M6G.

The behavioral evidence cited above is not a direct measure of neuronal activation and does not directly show functionally active CB_2 receptors in the brainstem. To determine whether CB_2 receptor agonists can activate neurons of the DMNX, we investigated the expression of phosphorylated extracellular signal–regulated kinase 1/2 (pERK) in rat DMNX neurons by immunohistochemistry (*14*), because phosphorylation of this enzyme is enhanced by cannabinoid agonists in other regions of the brain and in cell lines (*26*, *27*). Administration of the CB_2 receptor agonist AM1241 increased pERK in DMNX neurons when compared with vehicle-treated controls (Fig. 4).

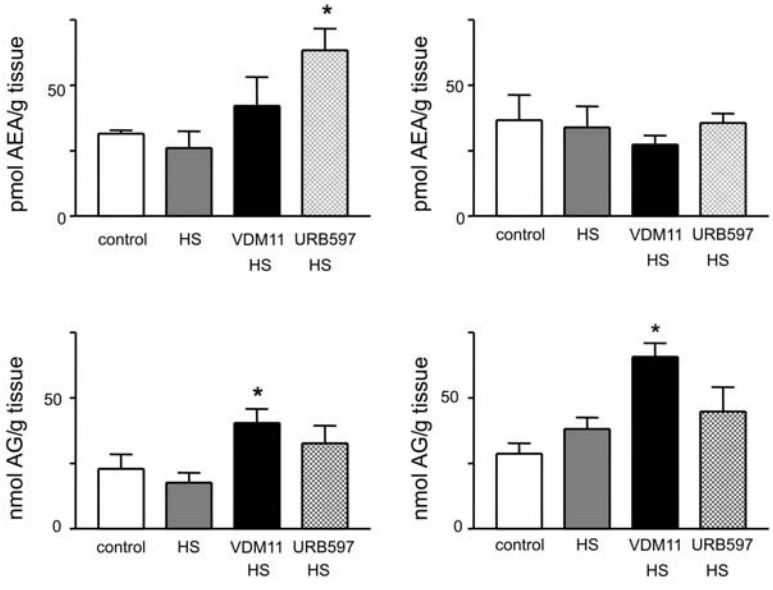

Fig. 3. Endocannabinoid levels in the ferret brainstem (**left**) and cerebellum (**right**). Control ferrets received intragastric normal saline ($n = 4$ to 7). After the emetic stimulus intragastric hypertonic saline (HS), levels were not significantly increased. URB597 (5 mg/kg) increased anandamide (AEA) levels in the brainstem but not in the cerebellum. VDM11 (3 mg/kg) increased total arachidonoyl glycerol (1-AG and 2-AG, 1AG accounts for about 20% and 2-AG about 80% of total) in both the brainstem and cerebellum when compared with the HS group ($P < 0.05$).

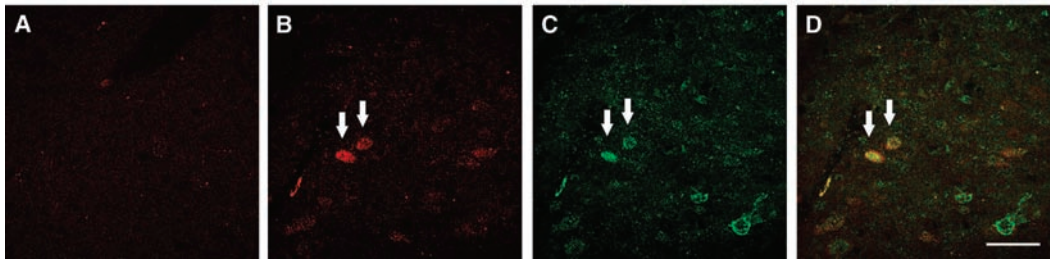

Fig. 4. A CB_2 receptor agonist activates neurons in the dorsal motor nucleus of the vagus (DMNX) of the rat. Immunoreactivity for pERK in rats treated with vehicle (**A**) ($n = 3$) and (**B**) the selective CB_2 receptor agonist, AM1241 (1 mg/kg; $n = 7$). AM1241 stimulated the expression of 5.6 ± 1.2 pERK immunoreactive cells per section in the DMNX compared with 1.2 ± 0.2 pERK immunoreactive cells in vehicle-treated animals. pERK immunoreactive cells were also observed in the nucleus of the solitary tract (not shown on figure). pERK immunoreactivity (red) was observed in nuclei and cytoplasm of activated cells. (**C**) CB_2 receptor immunoreactivity (green) was observed on the cell membrane and in the cytoplasm of DMNX neurons. (**D**) Overlay of pERK and CB_2 receptor illustrate the presence of pERK in neurons that express the CB_2 receptor (arrows). pERK immunoreactivity was observed in about 15 to 20% of the CB_2 immunoreactive neurons. Scale bar, 50 μm.

We have shown that CB_2 receptors are present in the brainstem and also in the cortex and cerebellum. As inferred by the use of a selective CB_2 antagonist, the brainstem receptors are functionally coupled to inhibition of emesis when costimulated with CB_1 receptors by an endogenous cannabinoid capable of activating both receptors. The extent of participation of CB_2 receptors in this effect is sufficient to reduce the widespread behavioral actions associated with the administration of CB_1 agonists. However, generalized activation of CB_2 receptors leads to immunosuppression (*28*) and is potentially deleterious if used as a therapy. Others have suggested that modulating the endocannabinoid system in the CNS represents a promising strategy for therapies for CNS disorders (*29*). Our observations suggest that targeting specific local populations of cannabinoid receptors (both CB_1 and CB_2) by enhancing endocannabinoid levels where they are released represents a therapeutic strategy that may be useful in disorders where either CB_1 or CB_2 receptor activation alone would not be desirable. This approach would circumvent the psychotropic and immunosuppressive side effects of exogenously administered cannabinoids and would provide an alternative approach for the therapeutic utilization of this unique neuroregulatory system.

References and Notes

1. V. Di Marzo, M. Bifulco, L. De Petrocellis, *Nat. Rev. Drug Discov.* **3**, 771 (2004).
2. A. C. Howlett *et al.*, *Pharmacol. Rev.* **54**, 161 (2002).
3. L. De Petrocellis, M. G. Cascio, V. Di Marzo, *Br. J. Pharmacol.* **141**, 765 (2004).
4. T. P. Dinh *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 10819 (2002).
5. T. F. Freund, I. Katona, D. Piomelli, *Physiol. Rev.* **83**, 1017 (2003).
6. T. W. Klein *et al.*, *J. Leukoc. Biol.* **74**, 486 (2003).
7. S. D. Skaper *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3984 (1996).
8. E. Nunez *et al.*, *Synapse* **53**, 208 (2004).
9. S. A. Golech *et al.*, *Brain Res. Mol. Brain Res.* **132**, 87 (2004).
10. C. Benito *et al.*, *J. Neurosci.* **25**, 2530 (2005).
11. C. Benito *et al.*, *J. Neurosci.* **23**, 11136 (2003).
12. G. Griffin, Q. Tao, M. E. Abood, *J. Pharmacol. Exp. Ther.* **292**, 886 (2000).
13. S. M. Brown, J. Wager-Miller, K. Mackie, *Biochim. Biophys. Acta* **1576**, 255 (2002).
14. Materials and methods are available as supporting material on *Science* Online.
15. I. Matias *et al.*, *Eur. J. Biochem.* **269**, 3771 (2002).
16. M. D. Van Sickle, Q. J. Pittman, K. A. Sharkey, unpublished observations.
17. A. V. Derbenev, T. C. Stuart, B. N. Smith, *J. Physiol.* **559**, 923 (2004).
18. M. D. Van Sickle *et al.*, *Gastroenterology* **121**, 767 (2001).
19. P. J. Hornby, *Am. J. Med.* **111** (suppl. 8A), 106S (2001).
20. L. De Petrocellis, T. Bisogno, J. B. Davis, R. G. Pertwee, V. Di Marzo, *FEBS Lett.* **483**, 52 (2000).
21. D. Fegley *et al.*, *J. Pharmacol. Exp. Ther.* **313**, 352 (2005).
22. M. D. Van Sickle, J. S. Davison, K. A. Sharkey, unpublished observations.
23. G. Marsicano *et al.*, *Science* **302**, 84 (2003).
24. S. Gaetani, V. Cuomo, D. Piomelli, *Trends Mol. Med.* **9**, 474 (2003).
25. J. P. Chhatwal, M. Davis, K. A. Maguschak, K. J. Ressler, *Neuropsychopharmacology* **30**, 516 (2005).
26. E. Valjent *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 491 (2005).
27. M. Bouaboula *et al.*, *Eur. J. Biochem.* **237**, 704 (1996).
28. T. W. Klein *et al.*, *J. Leukoc. Biol.* **74**, 486 (2003).
29. B. F. Cravatt, A. H. Lichtman, *Curr. Opin. Chem. Biol.* **7**, 469 (2003).
30. N. E. Buckley *et al.*, *Eur. J. Pharmacol.* **396**, 141 (2000).

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Fig. S1
References

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Observing Others: Multiple Action Representation in the Frontal Lobe

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Observation of actions performed by others activates monkey ventral premotor cortex, where action meaning, but not object identity, is coded. In a functional MRI (fMRI) study, we investigated whether other monkey frontal areas respond to actions performed by others. Observation of a hand grasping objects activated four frontal areas: rostral F5 and areas 45B, 45A, and 46. Observation of an individual grasping an object also activated caudal F5, which indicates different degrees of action abstraction in F5. Observation of shapes activated area 45, but not premotor F5. Convergence of object and action information in area 45 may be important for full comprehension of actions.

Understanding actions performed by others is a fundamental social ability. There is now wide consensus that the activation of the motor system is a necessary requisite for this ability. A mere visual representation, without involvement of the motor system, provides a description of the visible aspects of the movements of the agent, but does not give information critical for understanding action semantics, i.e., what the action is about, what its goal is, and how it is related to other actions (*1*, *2*). Action information, however, without knowledge about the identity of the object acted upon, is not sufficient to provide a full understanding of the observed action. Only when information about the object identity is added to the semantic information about the action can the actions of other individuals be completely understood (*3*).

The functional properties of a set of neurons in monkey ventral premotor cortex (area F5) provide evidence for the involvement of the motor system in action understanding. These "mirror" neurons discharge both when the individual performs an action and when the individual observes another person performing the same action (*4*, *5*). They therefore match the observed action with its internal motor representation. F5 neurons responding to the observation of grasping respond equally well when a piece of food or a solid object of similar size and shape is being grasped. The object's identity appears to be ignored in F5 (*4*, *5*).

We used fMRI in five awake monkeys (M1, M3 to M6) (*6*–*9*) to test how actions performed by others are represented in the monkey frontal lobe. In experiment 1, we intended to localize the frontal lobe regions involved in action observation. Monkeys saw video clips showing a full view of a person grasping an object ("acting person"), or an isolated hand grasping objects ("hand action") and static single frames or scrambled videos as controls. The acting person movies approximate the visual stimulation used in F5 single-cell studies (*4*, *5*) and provide context information that is lacking in the hand action movies, which has been used in most human

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