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Cannabinoids Reveal Separate Controls for Whisking Amplitude and Timing in Rats

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Pietr MD, Knutsen PM, Shore DI, Ahissar E, Vogel Z. Cannabinoids reveal separate controls for whisking amplitude and timing in rats. *J Neurophysiol* 104: 2532–2542, 2010. First published September 15, 2010; doi:10.1152/jn.01039.2009. Whisking is controlled by multiple, possibly functionally segregated, motor sensory-motor loops. While testing for effects of endocannabinoids on whisking, we uncovered the first known functional segregation of channels controlling whisking amplitude and timing. Channels controlling amplitude, but not timing, were modulated by cannabinoid receptor type 1 (CB1R). Systemic administration of CB1R agonist Δ^9 -tetrahydrocannabinol (Δ^9 -THC) reduced whisking spectral power across all tested doses (1.25–5 mg/kg), whereas whisking frequency was affected at only very high doses (5 mg/kg). Concomitantly, whisking amplitude and velocity were significantly reduced in a dose-dependent manner (25–43 and 26–50%, respectively), whereas cycle duration and bilateral synchrony were hardly affected (3–16 and 3–9%, respectively). Preadministration of CB1R antagonist SR141716A blocked Δ^9 -THC-induced kinematic alterations of whisking, and when administered alone, increased whisking amplitude and velocity but affected neither cycle duration nor synchrony. These findings indicate that whisking amplitude and timing are controlled by separate channels and that endocannabinoids modulate amplitude control channels.

INTRODUCTION

Active whisking and touch are complex functions that involve various motor and sensory variables (Ahissar and Zacksenhouse 2001; Berg and Kleinfeld 2003; Bermejo et al. 1998; Brecht et al. 1997; Carvell and Simons 1990; Diamond et al. 2008; Knutsen et al. 2006; Mitchinson et al. 2007; Semba and Komisaruk 1984; Vincent 1912; Welker 1964). While exploring and palpating objects, the rat motor system controls the amplitude, velocity, and duration of each whisking cycle as well as those of subcycle components, the rhythm and duration of each whisking bout, and the synchronization and coordination between different whiskers in both mystacial pads (Gao et al. 2003; Mitchinson et al. 2007). The neuronal circuits that are associated with whisking control are arranged in a complex network of parallel and nested motor-sensory-motor loops (Kleinfeld et al. 1999, 2006). Whether these networks control whisking variables collectively or whether different variables are controlled by different neuronal channels is not yet known.

Cannabis sativa (e.g., marijuana) is a source of medicinal and recreational psychotropic preparations (Croxford 2003; Earleywine 2002; Iversen 2003). The major psychoactive compound of *cannabis* is Δ^9 -tetrahydrocannabinol (Δ^9 -THC) (Mechoulam et al. 1970). Δ^9 -THC, and other structurally related compounds (termed cannabinoids), exert a range of physiological, behavioral, and cognitive effects (Abel 1970b; Compton et al. 1992; Isbell et al. 1967; Manno et al. 1970; Reeve et al. 1983; Wilson et al. 1994). Neuronal effects of cannabinoids are mediated by the G protein-coupled cannabinoid receptor type 1 (CB1R) (Devane et al. 1988; Matsuda et al. 1990), which is typically located on presynaptic terminals and inhibits neurotransmitter release when activated (Katona et al. 1999, 2000; Kreitzer and Regehr 2001).

The physiological roles of endocannabinoids are suggested in part by the specificity of CB1R expression throughout the brain. Whereas the basal ganglia, cerebellum, neocortex, and hippocampus contain high levels of CB1R (Egertova and Elphick 2000; Herkenham et al. 1990; Marsicano and Lutz 1999; Morales et al. 2007; Tsou et al. 1998), CB1R expression in the thalamus and brain stem is relatively sparse (Tsou et al. 1999). Furthermore, CB1R expression varies along the cortical surface and between cortical layers (Bodor et al. 2005; Lafourcade et al. 2007). The expression patterns of CB1R may explain sensory, motor, and cognitive effects of cannabinoids, such as modulation of hippocampal-dependent memory processes (Robbe et al. 2006; Sullivan 2000) and basal ganglia-related control of locomotion (Sanudo-Pena et al. 1999, 2000b; Shi et al. 2005).

The effects of exogenously administered cannabinoids and the presence of CB1 receptors throughout motor regions of the mammalian brain suggest that endocannabinoids are involved in motor control. By virtue of the closed-loop architecture of the vibrissal motor-sensory system, and the spatial specificity of CB1R expression, it is possible that endocannabinoids modulate behavior in a parameter-specific manner. Indeed, CB1R activation alters specific kinematic variables during lever pressing in rats (McLaughlin et al. 2000), and the CB1R antagonist SR141716A enhances the amplitude of sensory evoked activity in S1 (Patel et al. 2002). Here, we tested whether cannabinoid signaling is associated with specific kinematic alterations of whisking, as suggested by CB1R expression patterns in the whisker system and by known region-specific functional involvement in whisker movement control. Using high-speed video and systemic administration of CB1R agonists and antagonists, we found that the amplitude of

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spontaneous, free-air whisking in head-restrained rats was differentially modulated by CB1R activation or inactivation, suggesting that CB1R may be part of an endogenous signaling system involved in dynamically controlling whisking movements. Furthermore, the specific effect of CB1R activation and inactivation on whisking amplitudes suggests that amplitude is controlled separately from timing in this system. We propose that the anatomical nature of this functional separation can be explained in part by the spatial distribution of CB1R throughout the rodent brain.

METHODS

Drugs

Δ^9 -THC and SR141716A were obtained from the National Institute on Drug Abuse (Baltimore, MD). Δ^9 -THC from its ethanol stock solution of 100 mg/ml was mixed with vehicle, a PBS containing 25% (wt/vol) 2-hydroxypropyl-1- β -cyclodextrin (Cavazol W7 HP, Wacker), to a final concentration of 1 mg/ml (Jarho et al. 1996). SR141716 was diluted with a vehicle containing ethanol, surfactant (Cremophor, Sigma), and saline (0.9% NaCl) in a 1:1:18 mixture to produce a final concentration of 1 mg/ml. Drugs or vehicles were administered intraperitoneally (IP) at constant final volume, i.e., when a lower dose had to be injected the 1 mg/ml was diluted with the vehicle to give the desired dose always in a constant volume. The same vehicles were used for injections in control animals.

Animals

Female Wistar rats, 3–5 mo of age and weighing 200–300 g, served as subjects. Rats were housed individually on a 12:12-h light-dark cycle. Experimental sessions were run once a week or once every 2 wk.

Surgery

Rats were fitted with head mounts for head immobilization as previously described (Knutsen et al. 2008). Briefly, animals were anesthetized (pentobarbitone, 35 mg/kg, ip), the skull was exposed, and holes were drilled to fit stainless steel screws (MX-0080-02B, Small Parts, Miami Lakes, FL). Bone and screws were covered with 4-META-containing resin cement (Chemice II, Sun Medical, Moriyama, Japan) and acrylic cement (Jet Acrylic, Lang Dental Mfg, Wheeling, IL), in which two screws were embedded for head immobilization. Postoperative care included antibiotics (penicillin and streptomycin; Pen-Strep, 2 ml/kg, sc), analgesics (carprofen; 5 mg/kg, sc), and ad libitum food and water.

Drug administration schedule

Rats were injected intraperitoneally with Δ^9 -THC, SR141716A, or a combination of the two. When co-administered, SR141716A was injected 45 min before Δ^9 -THC. Vehicle controls mimicked drug administration schedules and their volumes. Experiments using different drugs and/or doses on the same rats were separated by a minimum of 7 days.

Measuring whisker movements

After at least 1 wk of surgical recovery, rats were manually handled for 30–60 min daily over a 2- to 3-wk period and gradually acclimatized to the experimental environment and head-fixing procedure. Small amounts of mango juice were given to the rats throughout these acclimatization sessions. Experimental sessions started when rats remained calm over a period of ≥ 20 min in the head-restraining device.

Although blood plasma levels of Δ^9 -THC drop rapidly (min), the drug can persist in fat and brain tissue for much longer (Kreuz and Axelrod 1973). Δ^9 -THC also has significant effects on the cortical EEG (Buonamici et al. 1982), hippocampal local field potentials, and locomotion several hours after injection (Hajos et al. 2008; Robbe et al. 2006). Therefore data acquisition sessions were repeated in the first, fourth, and seventh hours after drug administration. In each sampling interval, whisking was recorded for 30 min while rats were inserted into a plastic tube and head restrained. Rats were securely fixed in place by an articulated manipulator (NF60101, Noga Engineering, Schlomi, Israel), which was attached to two mounting screws cemented to the skull (Fig. 1A).

Spontaneous whisker movements were captured at 1,000 frames/s at 640×640 pixels using a high-speed video system (MotionScope PCI, Redlake, San Diego, CA) positioned above the rat. The video acquisition system has previously been described by Knutsen et al. (2005). In each acquisition session (30 min), 5–12 movies ≤ 13 s in duration were acquired. Whisker tracking was facilitated by removing most whiskers during brief (5–10 min) isoflurane anesthesia, leaving only the C2 whisker on each side of the face. Small drops of metallic marker dye (Artline 990XF, Shachihata, Nagoya City, Japan) were applied at two locations along the intact whisker shafts, separated by 15–20 mm (Fig. 1A). Movements of these dye labels were tracked off-line, using the MATLAB based *WhiskerTracker* application (<http://code.google.com/p/whiskertracker>). Whisker angles in individual frames were defined by the angle of a line drawn through the two locations of the dye labels with respect to the eye–nose axis (Fig. 1B), such that a retracted whisker parallel to this line has zero angle. All experimental procedures were conducted in accordance with National Institutes of Health guidelines and with approval of the Weizmann Institute of Science Institutional Animal Care and Use Committee.

Kinematic and spectral analysis of whisking

Whisking movements were analyzed during bouts of continuous free-air whisking. Whisking bouts were manually selected from time series of tracked whisker movements. Individual high-speed movies contained one or more bouts (Fig. 1C). Individual protraction–retraction cycles were extracted by an automatic peak detection algorithm that identified protraction onsets, peak protractions, and peak retractions. These events were used to determine the amplitude, duration, and peak velocity of individual protraction and retraction movements (Fig. 1D).

Power spectral densities (PSDs) of angular velocity were estimated for each bout of whisking using Welch's averaged, modified periodogram method as implemented in the MATLAB function *pwelch* (Fig. 1E). These PSDs measures the distribution of frequencies and the power at each frequency, with the power reflecting the intensity or amplitude of whisking at each frequency. Spectrograms of whisking bouts were computed by dividing individual bouts into overlapping segments windowed with a Kaiser window and calculating the PSD of 350-ms segments in steps of 20 ms. The instantaneous whisking frequency was estimated from the mode of the PSD in each segment. The spectral purity of whisking was estimated by averaging all PSD windows from the periodogram of a particular bout and normalizing the result by the whisking bout duration. We define the whisking bout frequency as the mode of the averaged periodogram (f_0). The spectral purity of whisking is expressed by the spectral width (Δf_0), corresponding to the half-width of the PSD at half-amplitude of f_0 (Fig. 1F). Whisking power was estimated as the area under the averaged periodogram in the range 2–12 Hz.

Statistical analysis

Biometric whisking parameters were examined across the population of rats according to standard methods for within-subject design (Loftus and Masson 1994). The significance of effects induced by

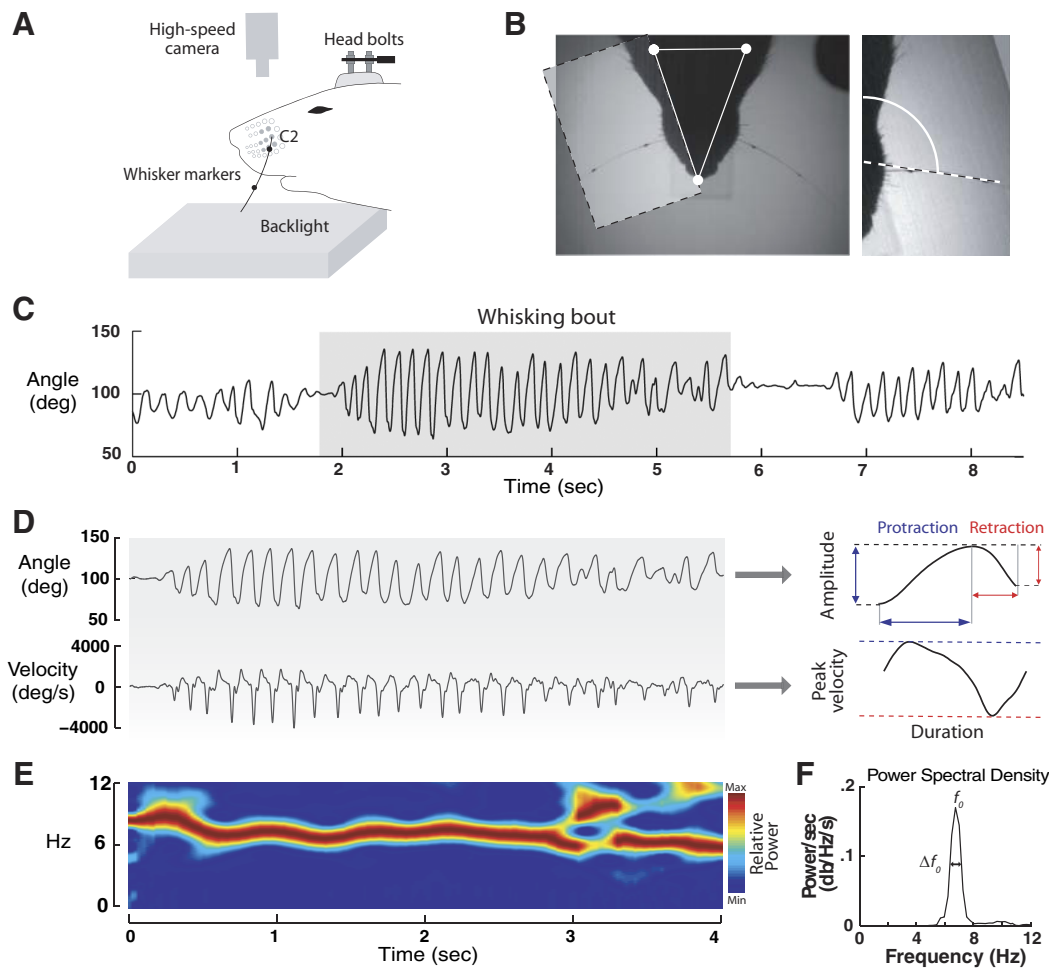


FIG. 1. Kinematic and spectral analysis of whisker movements. *A*: experimental setup for measuring spontaneous whisker movements in awake, head-restrained rats. A high-speed video camera was used to simultaneously image whiskers on both sides of the head. Whisker visibility was enhanced by illuminating with a strobed backlight. Whiskers of interest were marked with small drops of metallic marker dye at 2 locations. *B*: head position was marked manually (white dots), and a rectangular region of interest (dashed rectangle) parallel to a line intersecting the ipsilateral eye and nose was extracted. In each frame, whisker labels were automatically tracked in head-centered coordinates and a line connecting the labels used as an approximation of whisker location (white dashed line). *C*: time-varying trace of right C1 whisker angle throughout a 8.5-s video capturing several episodes of whisking (bouts). The gray box indicates the duration of a single bout of whisking (3.8 s). *D*: only whisker movements occurring within whisking bouts were selected for further analysis. Individual whisk cycles were automatically extracted from a bout and characterized by duration (horizontal arrows), amplitude (vertical arrows), and peak velocity (dotted lines) for protraction (blue) and retraction (red). *E*: the continuous spectrogram of whisking velocity (the 1st derivative of whisker angle) was computed in piecewise steps using Welch's averaged, modified periodogram method. *F*: all time bins of spectrogram were averaged to obtain the power spectral density (PSD). The whisking frequency (f_0) was defined as the mode of the PSD. The whisking power corresponds to the area under the PSD curve in the range 2–12 Hz. The spectral purity of whisking was assessed by the spectral width (Δf_0), defined as the half-width at half-maximum of the PSD curve. In the example shown, f_0 was 7.1 Hz and Δf_0 was 0.6 Hz.

different drug treatments was evaluated by a two-way repeated-measures ANOVA (RM-ANOVA), followed by Fisher's protected least significant difference (Fisher's PLSD) test for multiple comparisons. *F* values were compared with Huynh-Feldt epsilon-corrected *F* values to detect departures from ANOVA assumptions of sphericity. Thus in all instances detected, we report the corrected probability value as *Phf* instead of *P*.

RESULTS

Differential effects of Δ^9 -THC on whisking power and frequency

For each video recording of whisker movements, we measured the whisker angles relative to the mystacial pad and extracted continuous epochs of motion (Fig. 1, *A–C*). Whisking within each epoch was characterized by a stable frequency

(Fig. 1, *D* and *E*), in agreement with previous reports on head-restrained rats (Berg and Kleinfeld 2003; Gao et al. 2001b). No significant differences in frequencies between left- and right-side whiskers were observed. Thus unless mentioned specifically, all analysis was restricted to movements of whiskers on one side of the snout (usually the right).

Systemic administration of Δ^9 -THC (1.25, 2.5, and 5 mg/kg) significantly attenuated spontaneous whisking movements in all rats in a monotonic, dose-dependent manner. Typical whisking patterns within the fourth hour after control injection of vehicle or increasing doses of Δ^9 -THC are shown in Fig. 2, *A* and *B*, for two different rats. Although Δ^9 -THC induced a clear reduction in whisker movement amplitudes, the number of whisk cycles per second did not change. To quantify this, we computed the PSD for individual whisking epochs (Fig. 2, *C* and *D*). The PSDs showed a clear dose-dependent reduction in

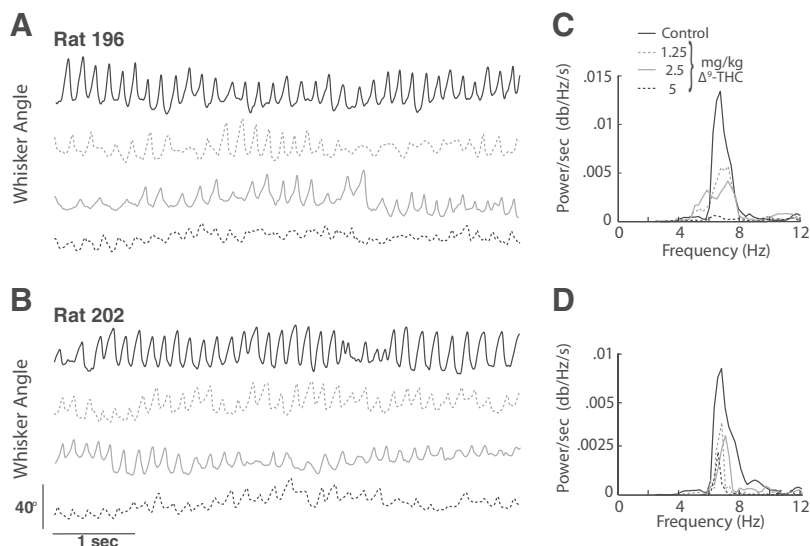


FIG. 2. Effects of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) on spontaneous whisking patterns. *A* and *B*: whisker movements (angles) executed during representative bouts by 2 different rats (rats 196 and 202; each bout plotted for 6 s). Protractions and retractions are plotted as upward and downward deflections, respectively. All measurements were made within the 4th hour after injection of vehicle or Δ^9 -THC at indicated concentrations. *C* and *D*: PSDs computed for the whisking traces of *A* and *B* (rats 196 and 202, respectively).

power (Fig. 2, *C* and *D*) but no change in the average or spread of the frequency distribution. In agreement with previous reports (Gao et al. 2001b), PSDs typically peaked between 6 and 8 Hz, regardless of the Δ^9 -THC dose administered (Fig. 2, *C* and *D*).

To test the significance of Δ^9 -THC effect on whisking kinematics, we computed PSDs for each whisking bout. For each PSD, we measured the power of whisking (area under the PSD between 2 and 12 Hz) and the mode frequency (the peak of the PSD). For each drug dose (control, 1.25, 2.5, and 5 mg/kg) and acquisition time point (1st, 4th, and 7th hour), we averaged all power and frequency measurements obtained for any single rat ($n = 6$). These averages were compared using RM-ANOVA with dose and time after injection as factors. For

each Δ^9 -THC dose, there was a clear reduction in net power of whisking [$F(3,15) = 11.26$, $Phf < 0.01$, $\epsilon = 0.84$, $MSe = 0.003$; Fig. 3*A*]. There was also a trend for reduced power across the three acquisition times tested [$F(2,10) = 11.26$, $P = 0.05$, $MSe = 0.001$], consistent with previous observations (Gao et al. 2001b). The interaction between dose and acquisition time was not significant [$F(6,30) = 1.21$, $Phf = 0.3$, $\epsilon = 0.57$, $MSe = 0.001$]. Post hoc analysis indicated that the whisking power was significantly attenuated across all doses and time points and on average (across all time points) decreased by 64–87% for Δ^9 -THC doses of 1.25–5 mg/kg, respectively (Fig. 3*A*).

Overall, changes in whisking frequency were minor and were reduced by a significant value only at the highest dose (5

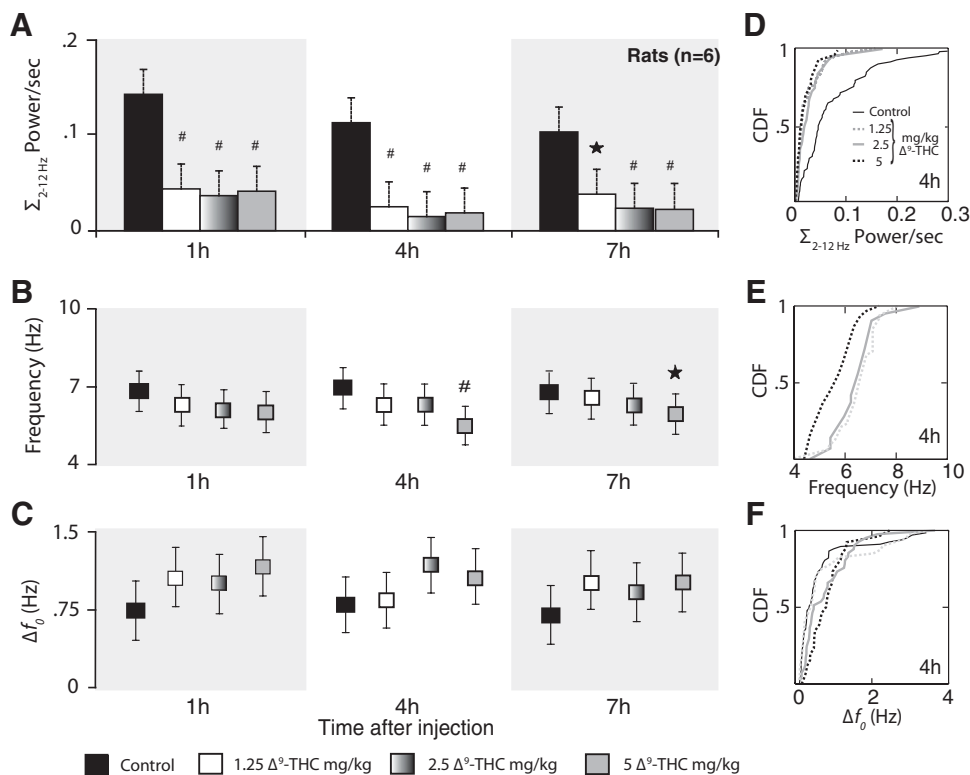


FIG. 3. Δ^9 -THC-mediated changes in spectral properties of whisker movements. *A*: bar graphs of net whisking power, computed for individual whisking bouts executed within the 1st, 4th, and 7th hour after Δ^9 -THC injection ($n = 6$ rats). Δ^9 -THC induced a robust reduction in whisking power at all tested concentrations. *B* and *C*: distributions of whisking frequencies and spectral widths computed for the same data as in *A*. Boxes indicate mean values and error bars the 95% CI computed from the within-subject MSE term of the ANOVA at the level of relevant interaction. Fisher's protected least significant difference (PLSD) post hoc test for multiple comparisons was performed within every time point after injection of each Δ^9 -THC dose vs. vehicle control. * $P < 0.05$ and # $P < 0.01$. *D*–*F*: cumulative probability distribution functions (CDFs) of (*D*) whisking power, (*E*) whisking frequencies, and (*F*) spectral widths. The number of whisking bouts included in all analysis, across all 6 rats, was 75 (control), 48 (Δ^9 -THC 1.25 mg/kg), 44 (Δ^9 -THC 2.5 mg/kg), and 32 (Δ^9 -THC 5.0 mg/kg).

mg/kg) in the fourth and seventh hours after injection [$F(3,15) = 9.63$, $P < 0.01$, $MSe = 0.348$; Fig. 3B]. A reduction of 1.4 Hz, after 5 mg/kg Δ^9 -THC, was the maximal shift in whisking frequencies. We did not observe any effect on whisking frequency either as a function of the acquisition time [$F(2,10) = 3.44$, $Phf = 0.09$, $\varepsilon = 0.75$, $MSe = 0.146$] or as a dose \times time interaction [$F(6,30) = 0.85$, $P = 0.54$, $MSe = 0.187$].

The instantaneous whisking frequency remained relatively constant within bouts of whisking (Berg and Kleinfeld 2003; Gao et al. 2001b). This high spectral fidelity, quantified by the spectral width of the PSD (Δf_0 ; Fig. 1F), was not affected by Δ^9 -THC dose [$F(3,15) = 2.75$, $P = 0.08$, $MSe = 0.122$; Fig. 3C] or by the acquisition time [$F(2,10) = 0.23$, $Phf = 0.09$, $\varepsilon = 0.75$, $MSe = 0.146$]. In addition, we found no interactions between dose and time [$F(6,30) = 1.03$, $P = 0.4$, $MSe = 0.054$].

We validated our RM-ANOVA with nonparametric [Kolmogorov-Smirnov (K-S)] comparisons between the cumulative probability functions (CDFs) of whisking power and frequency obtained from all rats in the fourth hour after injection of Δ^9 -THC. At all tested Δ^9 -THC doses, whisking power was significantly attenuated compared with control at the 95% rejection limit (Fig. 3D). Whisking frequency, however, was significantly affected only at the highest Δ^9 -THC dose of 5 mg/kg (Fig. 3E). In contrast to the RM-ANOVA, nonparametric testing showed a significant increase of spectral widths (i.e., a larger scatter of whisking frequencies) at the highest Δ^9 -THC dose in the fourth hour after injection ($P = 0.002$; Fig. 3F).

Whisking activity recovered to control levels, and no effects of acute Δ^9 -THC treatment were detected on any measured kinematic parameter 24 h after injection of 5 mg/kg Δ^9 -THC (data not shown).

Differential effects of Δ^9 -THC on whisk amplitude and duration

The differential effects of Δ^9 -THC on whisking power versus frequency could be caused by a temporal disruption of whisking cycles, such as a reduction of the whisk duty cycle, which could thereby shorten and possibly slow down the high-velocity retraction phase. To characterize whether such, or other similar, temporal effects were taking place, we compared the effects of Δ^9 -THC on whisking amplitudes and durations separately for whisker protractions and retractions (Fig. 4).

In control experiments, protraction and retraction amplitudes were 34.6 ± 5.9 and $34.3 \pm 5.9^\circ$, respectively. In Δ^9 -THC-injected animals, protraction and retraction amplitudes were reduced by an average of 9° (25% reduction) at 1.25 mg/kg to 15° (43%) at 5 mg/kg. Using RM-ANOVA, we found that Δ^9 -THC led to a significant, and dose-dependent, reduction of both protraction [$F(3,15) = 11.26$, $Phf < 0.01$, $\varepsilon = 0.56$, $MSe = 67.10$] and retraction [$F(3,15) = 11.26$, $Phf < 0.01$, $\varepsilon = 0.54$, $MSe = 66.66$] amplitudes (Fig. 4, A and B). These amplitude reductions were significantly attenuated with increasing time after injections, both with respect to protraction [$F(2,10) = 1.22$, $Phf = 0.26$, $\varepsilon = 0.55$, $MSe = 28.15$] and retraction [$F(2,10) = 6.28$, $P = 0.02$, $MSe = 14.99$]. We found no significant dose \times time interaction for protractions [$F(6,30) = 2.00$, $P = 0.15$, $MSe = 16.35$] or for retractions [$F(6,30) = 2.09$, $P = 0.12$, $MSe = 24.12$]. Post hoc analysis showed that

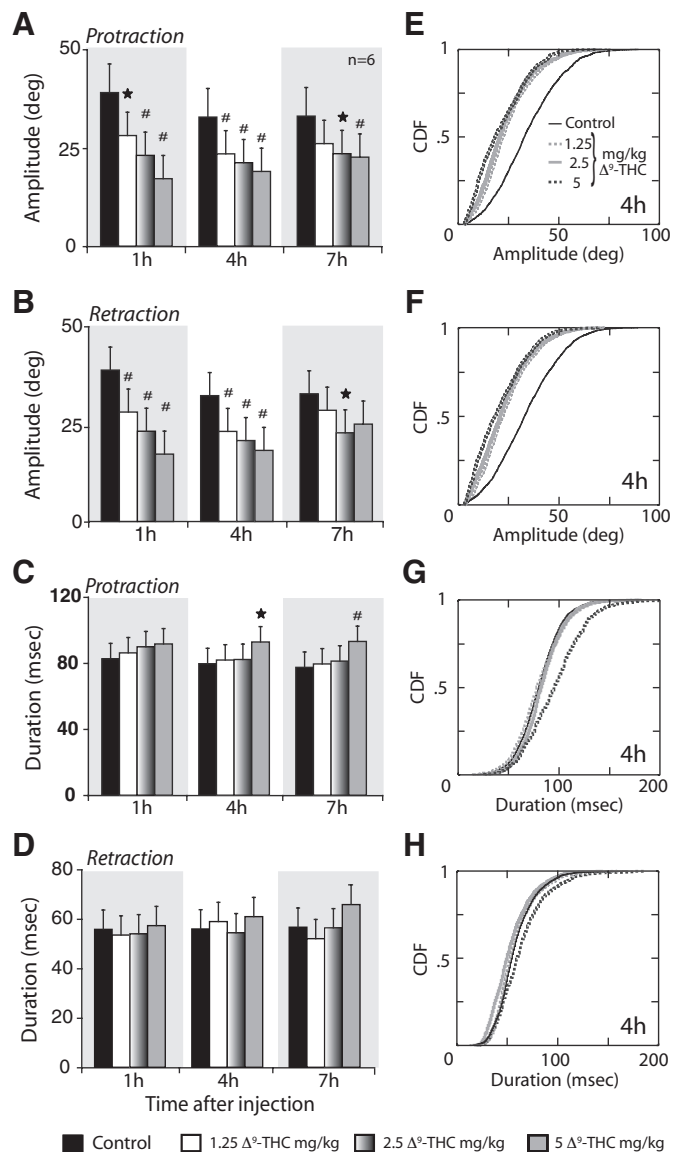


FIG. 4. Δ^9 -THC-mediated changes in kinematic properties of whisker protractions and retractions. A and B: average protraction and retraction amplitudes ($n = 6$ rats). C and D: average protraction and retraction durations. Bars indicate the means, and error bars indicate the 95% CIs computed from the within-subject MSE term of the ANOVA. Fisher's PLSD post hoc test for multiple comparisons was carried out for every time point and for each Δ^9 -THC concentration vs. control. * $P < 0.05$ and # $P < 0.01$. E and F: CDFs of protraction and retraction amplitudes in the 4th hour after administration of vehicle ($n = 1,186$), 1.5 mg/kg Δ^9 -THC ($n = 1,146$), 2.5 mg/kg Δ^9 -THC ($n = 1,029$), and 5 mg/kg Δ^9 -THC ($n = 588$ whiskers). CDFs obtained with all drug treatments were significantly different from the control CDF at the 95% rejection limit (Kolmogorov-Smirnov test). G and H: CDFs of protraction and retraction durations. Only the CDFs obtained after administration of 5 mg/kg Δ^9 -THC were significantly different from the control CDF at the 95% rejection limit (Kolmogorov-Smirnov test).

Δ^9 -THC effects were somewhat weaker during the seventh hour after injection, suggesting some drug excretion or desensitization.

Effects of Δ^9 -THC on the durations of the protraction and of the retraction were very small and followed a different dose-response curve (Fig. 4, C and D). In control experiments, protraction and retraction durations were 79.5 ± 9.3 and 54.7 ± 2.1 ms, respectively. In Δ^9 -THC-injected animals, protraction du-

ration increased by an average of 12 ms (16%) at 5 mg/kg. Although small, the effect of Δ^9 -THC dose on protraction duration was significant [$F(3,15) = 4.62$, $Phf = 0.04$, $\epsilon = 0.64$, $MSe = 113.93$] but only for the highest dose (Fisher's post hoc test). The acquisition time after injection had a significant influence on protraction duration [$F(2,10) = 4.57$, $Phf = 0.04$, $\epsilon = 0.96$, $MSe = 31.63$], but this was not modulated by the dose [$F(6,30) = 2.00$, $P = 0.15$, $MSe = 16.35$; Fig. 4B]. There was no significant change in retraction duration [$F(3,15) = 2.71$, $Phf = 0.12$, $\epsilon = 0.64$, $MSe = 81.05$], or acquisition time [$F(2,10) = 1.65$, $Phf = 0.24$, $\epsilon = 0.87$, $MSe = 40.51$] at all Δ^9 -THC doses.

We validated the RM-ANOVA results with nonparametric (K-S) comparisons between the CDFs of whisk amplitudes and durations measured for all rats in the fourth hour after Δ^9 -THC injection. In agreement with the effect on power of whisking, both protraction and retraction amplitudes were significantly decreased after Δ^9 -THC administration at any dose (Fig. 4, E and F); only protraction duration was significantly changed (increased) and only at the highest Δ^9 -THC dose (5 mg/kg; Fig. 4, G and H).

Δ^9 -THC-mediated amplitude reduction is accompanied by decrease in peak velocity

Amplitude reductions were accompanied by reductions in peak velocity (Fig. 5, A and B). In control animals, the average peak velocity during protraction and retraction was 769 ± 129 and $1432 \pm 299^\circ/\text{s}$, respectively. In the fourth hour after Δ^9 -THC administration, protraction peak velocity reduced by 26% at 1.25 mg/kg and by 50% at 5.0 mg/kg. RM-ANOVA analysis showed a significant effect of dose [$F(3,15) = 21.20$, $Phf < 0.01$, $\epsilon = 0.91$, $MSe = 21,997$; $F(3,15) = 13.00$, $Phf < 0.01$, $\epsilon = 0.63$, $MSe = 118,673$], a marginally significant effect of time [$F(3,15) = 4.66$, $Phf = 0.06$, $\epsilon = 0.75$, $MSe = 8,090$; $F(3,15) = 6.64$, $Phf < 0.05$, $\epsilon = 0.83$, $MSe = 35,593$], and no interaction between the two factors [$F(6,30) = 1.40$, $P = 0.25$, $MSe = 8,401$; $F(6,30) = 1.53$, $Phf = 0.20$, $\epsilon = 0.72$, $MSe = 50,363$] on peak protraction and retraction velocities, respectively. Although protraction peak velocities were significantly reduced at all doses and time points, retraction peak velocities were reduced at all doses and all but one time-point (7 h). This marginally weaker effect on retraction peak velocity could be

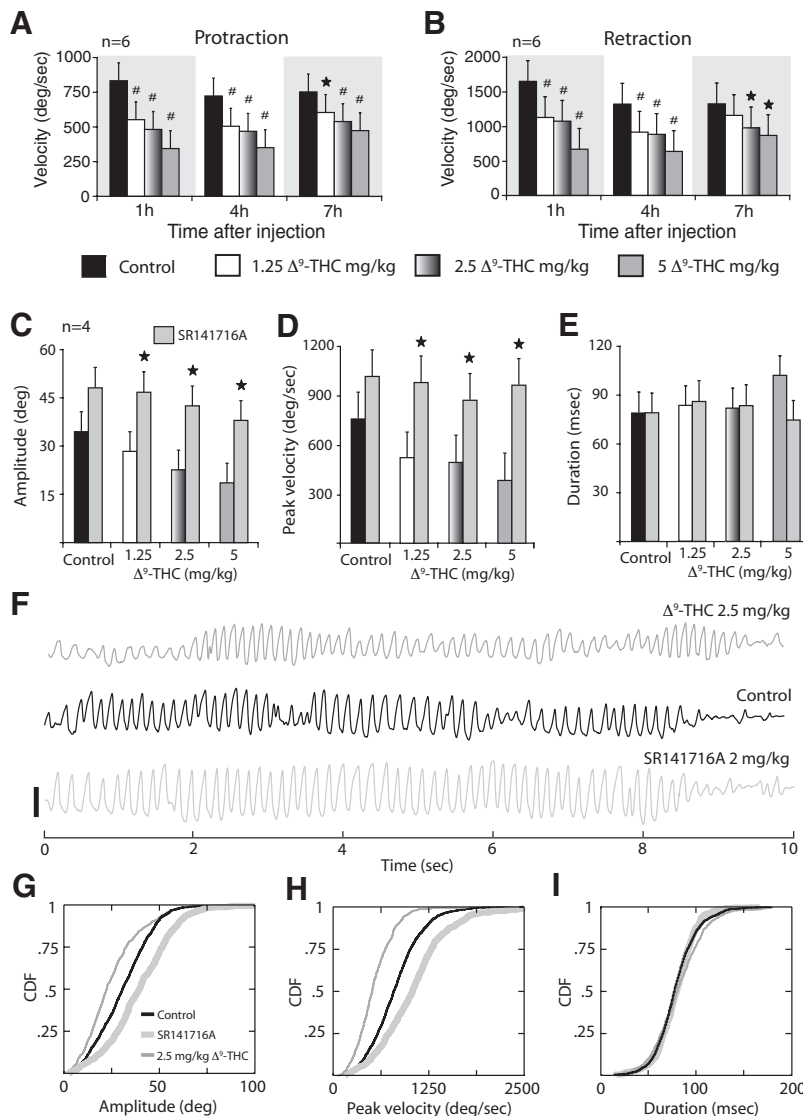


FIG. 5. Effects of a cannabinoid receptor type 1 (CB1R) agonist and antagonist on whisking kinematics. A and B: the bar graph represents mean protraction and retraction peak velocities for a group of subjects ($n = 6$). Error bars displayed at the 95% CI calculated from the within-subject MSe term of the ANOVA at the level of relevant interaction. Fisher's PLSD post hoc test for multiple comparisons was carried out within every time point for each Δ^9 -THC dose vs. control. $*P < 0.05$ and $\#P < 0.01$. C–E: effects of SR141716A (light gray bars) on protraction amplitudes (C), peak velocity (D), and duration (E) compared with control (black bars) and Δ^9 -THC administered at different doses 4 h after injection. Thick bars indicate means, and error bars are 95% within-subject CIs as reported by repeated measures (RM)-ANOVA. Fisher's PLSD post hoc test was performed separately for each Δ^9 -THC dose between SR141716A and vehicle pretreatment. $*P < 0.05$. F: typical traces of whisker angle executed by the same rat in the 4th hour after administration of Δ^9 -THC (2.5 mg/kg; dark gray), vehicle (black), or SR141716A (2 mg/kg; light gray). Vertical calibration bar corresponds to 50° . G–I: CDFs of protraction amplitudes (G), peak velocities (H), and durations (I) for control ($n = 1886$ whisks), Δ^9 -THC ($n = 564$ whisks), and SR141716A ($n = 592$ whisks) treatments.

caused partly by the passive component of whisker retraction (Berg and Kleinfeld 2003).

Δ^9 -THC modulates whisking via CB1 receptors

To determine the involvement of CB1 receptors in Δ^9 -THC effects on whisking behavior, we administered the selective CB1R antagonist SR141716A (Rinaldi-Carmona et al. 1994) 45 min before the administration of Δ^9 -THC or control vehicle and measured whisking behavior in the fourth hour after Δ^9 -THC/vehicle injection ($n = 4$). Reductions in protraction amplitudes and peak velocities caused by Δ^9 -THC were completely abolished by SR141716A (Fig. 5, C and D). RM-ANOVA analysis showed a significant effect on protraction amplitude of Δ^9 -THC dose [$F(3,9) = 11.03$, $Phf < 0.01$, $MSe = 22.80$] and SR141716A [$F(1,3) = 23.55$, $P < 0.05$, $MSe = 109.28$], but not of the interaction between these two factors [$F(2, 6) = 0.55$, $P = 0.66$, $MSe = 29.95$]. Moreover, we found a significant effect of SR141716A treatment on protraction velocity [$F(2,3) = 22.40$, $P < 0.05$, $MSe = 62,097$] but no effect of the Δ^9 -THC \times SR141716A interaction [$F(2, 6) = 1.81$, $Phf = 0.21$, $MSe = 19,884$]. Fisher's post hoc test performed for each Δ^9 -THC dose showed that SR141716A pretreatment completely counteracted Δ^9 -THC-mediated reductions in protraction amplitudes (Fig. 5C) and peak veloci-

ties (Fig. 5D). The same effects were observed for retraction phase of the whisk cycle (data not shown).

The increase in protraction duration observed with 5 mg/kg Δ^9 -THC (Fig. 4C) was completely abolished by SR141716A pretreatment (Fig. 5E). RM-ANOVA showed no significant effects on protraction duration of Δ^9 -THC dose [$F(1,3) = 1.28$, $Phf = 0.34$, $MSe = 96.44$], SR141716A treatment [$F(2,6) = 2.74$, $P = 0.19$, $MSe = 105.25$], or the Δ^9 -THC \times SR141716A interaction [$F(2, 6) = 4.72$, $Phf = 0.07$, $MSe = 87.28$].

Blocking endogenous cannabinoids increases whisking amplitude and does not affect whisking duration

By comparing individual epochs of whisking, it was evident that Δ^9 -THC or SR141716A applied alone affected whisker movement amplitudes in opposite directions (Fig. 5F). The same animals that were treated with SR141716A ($n = 4$) were also injected with Δ^9 -THC on other days. As was the case in other experiments (Figs. 2–5), Δ^9 -THC treatment resulted in a significantly decreased protraction amplitudes (40%; Fig. 5G) and peak velocities (36%; $P < 0.01$, K-S test; Fig. 5H), as well as in a minor, but significant, increase in protraction duration (4%; $P < 0.01$, K-S test; Fig. 5I). In contrast, SR141716A at 2 mg/kg significantly increased the protraction amplitudes by 41% (14°) and the protraction peak velocities by 30% relative

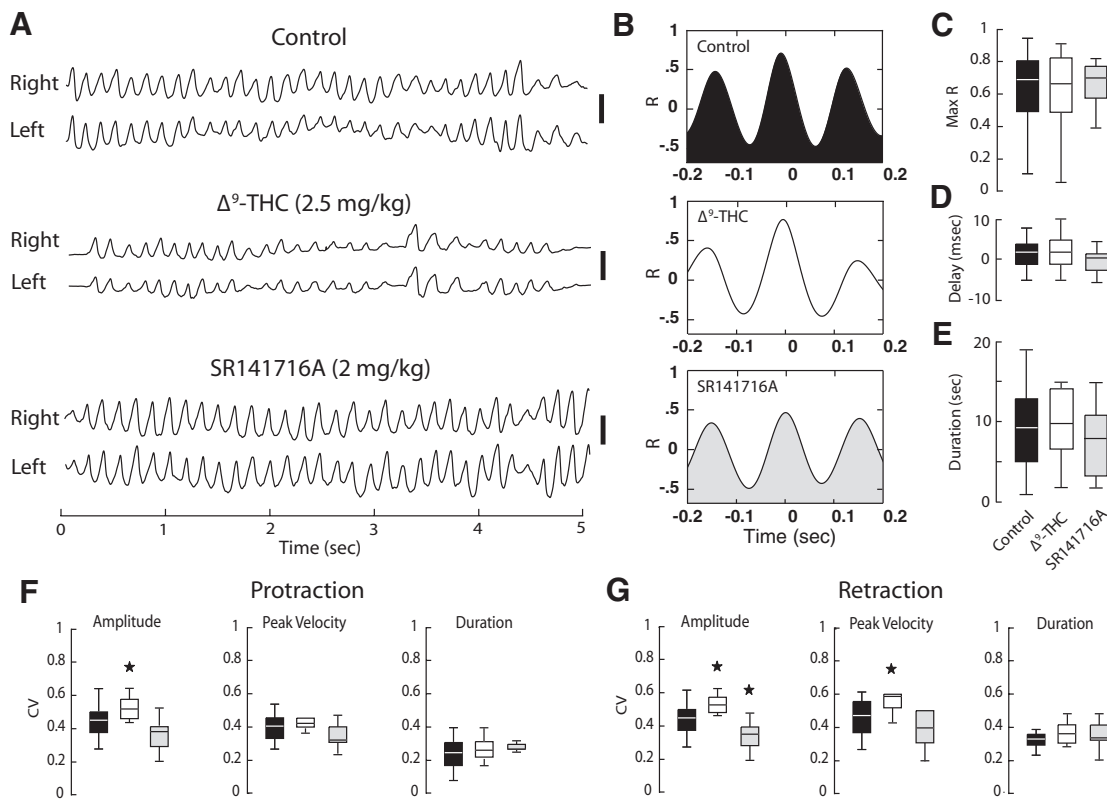


FIG. 6. Effects of CB1R agonist and antagonist on whisking coordination. *A*: right and left C2 whisker movements executed simultaneously (by the same rat) in the 4th hour after administration of vehicle, Δ^9 -THC (2.5 mg/kg), or SR141716A (2 mg/kg). Calibration bar corresponds to 50° angle. *B*: cross-correlations of the angle traces in *A*. Peaks were always centered close to 0, showing a high degree of synchrony between right and left whiskers, regardless of drug treatment. *C*: distributions of peak correlation coefficients obtained from cross-correlations between right and left whisker movements, such as those in *A* and *B*, executed in the 4th hour after administration of vehicle ($n = 36$ whisking bouts), Δ^9 -THC (2.5 mg/kg, $n = 29$), or SR141716A (2 mg/kg, $n = 16$). *D*: distributions of temporal delays of the peak correlation coefficients obtained from cross-correlations. *E*: distributions of whisking bout durations. None of the differences in *C–E* were significantly different ($P > 0.05$; 1-way ANOVA). *F*: distributions of within-bout CVs of amplitude, peak velocities, and durations for protraction and (*G*) retraction phase of the whisk cycle [$*P < 0.05$; 1-way ANOVA followed by Tukey-Kramer honestly significant difference (HSD) post hoc test]. Box plots in *C–G* denote lower and upper quartiles (gray box), medians (horizontal line inside boxes), and range of data (vertical lines).

to control experiments ($P < 0.05$, K-S test; Fig. 5, *C, D, G*, and *H*). However, SR141716A administration did not significantly affect protraction durations ($P = 0.18$; K-S test; Fig. 5*I*).

Cannabinoids do not affect duration, synchrony, and coordination of whisking bouts

We examined whether Δ^9 -THC or SR141716A affected the bilateral control of whisker movements. Free-air whisker movements of right and left whiskers were typically synchronous and amplitude coordinated in control animals (Fig. 6*A*). We found that, regardless of treatment (Δ^9 -THC or SR141716A), bilateral whisker movements remained temporally synchronous (Fig. 6*B*), as indicated by high correlations (Fig. 6*C*) and small time lags of peak correlations between movements of different whiskers (Fig. 6*D*). Cross-correlations between the same whisker on the left or right side always peaked close to zero and averaged ± 9 ms ($r = 0.45$ – 0.75 ; Fig. 6*D*). In our analysis, time lags above zero imply that the right whisker was phase-leading the left whisker. Peak cross-correlation coefficients, regardless of treatment condition, were distributed across a broad range of values with sample medians varying between 0.6 and 0.8 (Fig. 6*C*).

Amplitude coordination was estimated by the instantaneous angle difference divided by the average angle of right and left whiskers

$$(\Phi_{\text{LEFT}} - \Phi_{\text{RIGHT}}) / [(\Phi_{\text{LEFT}} + \Phi_{\text{RIGHT}}) / 2]$$

This formula expresses the ratio of the angle difference relative to the absolute angle amplitude and thus is an estimate

of motor error (assuming the target is to produce identical movements on both sides). The frame-by-frame, instantaneous measurements of amplitude coordination were averaged across all frames in each trial, and the distributions of such averages were compared between the different drug administration conditions. The results of these comparisons show that, in control animals, the motor error was $19.5 \pm 0.9\%$ and in Δ^9 -THC- or SR141716A-injected animals was $19.7 \pm 0.7\%$. These small differences were not significantly different ($P > 0.7$; 1-way ANOVA).

Whisking epochs ranged from short (< 1 s) to tens of seconds long and were on average 8.3 ± 0.8 s long in control animals. We did not observe any significant change in bout durations in the fourth hour after administration during either Δ^9 -THC or SR141716A treatments (1-way ANOVA; Fig. 6*E*).

Finally, we examined whether cannabinoid treatment affected variability of whisking kinematics (Fig. 6, *F* and *G*). We found that protraction amplitudes were more irregular, expressed by CVs, 4 h after treatment with 2.5 mg/kg Δ^9 -THC ($P < 0.05$, 1-way ANOVA; Fig. 6*F*). Within-bout fluctuations of protraction peak velocity and duration were unaffected regardless of treatment compared with vehicle controls. Variability of retraction amplitude was affected in the fourth hour after either Δ^9 -THC or SR141716A administration (Fig. 6*G*). SR141716A treatment, however, exerted an opposite effect to the one by Δ^9 -THC by reducing within-bout fluctuations of retraction amplitudes with respect to control. In addition to increasing irregularity of retraction amplitudes, Δ^9 -THC affected peak retraction velocities.

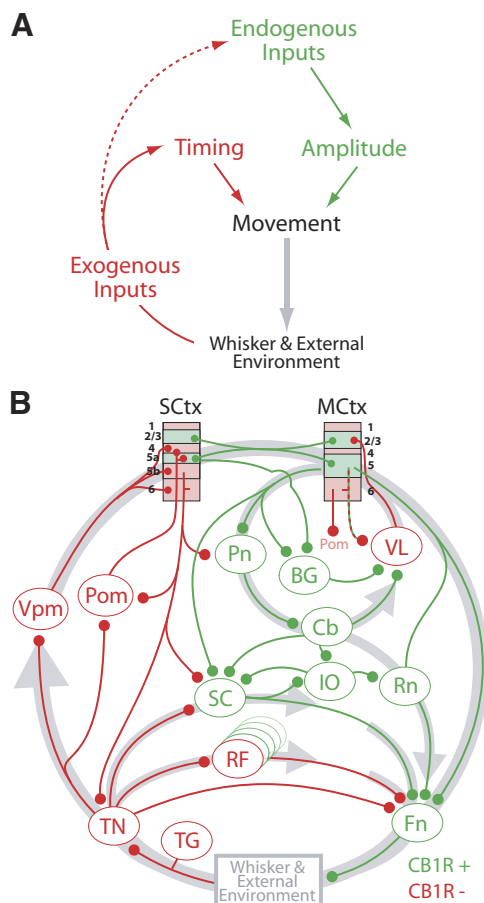


FIG. 7. Whisking is controlled by different signal sources. *A*: during free-air whisking, amplitude can be modulated by endogenous signals (i.e., signals generated internally) and does not require exogenously derived signals, such as contact and proprioception. However, timing of whisker movements is modulated during contact-dependent tasks and when proprioceptive signals change (e.g., restrained vs. unrestrained). Thus timing control is not affected by endogenous signals but its change requires exogenous signals, such as those generated by head/body movement or contact. *B*: CB1R is primarily expressed in motor circuits involved in control of whisker movement (CB1R+; green). CB1R expression is abundant, but highly regional or layer specific, throughout the rat nervous system. CB1Rs are absent in sensory regions that process whisker-related sensory inputs (CB1R-; red). Cells in trigeminal ganglion (TG) express CB1Rs, but primarily those located in the maxillary and non-vibrissal representation of the mandibular division of the TG (Price et al. 2003). Cells in the trigeminal nuclei (TN), the ventroposteromedial complex (Vpm), and posteromedial thalamic nucleus (Pom) of the thalamus do not express CB1Rs (Tsou et al. 1998). The primary somatosensory (S1) and motor (M1) cortices express CB1Rs primarily in layers 2/3 and 5a (Bodor et al. 2005; Eggen and Lewis 2007; Hill et al. 2007; Trettel et al. 2004). CB1R is not expressed in layer 5b of S1. The basal ganglia contain high levels of CB1R (Egertova and Elphick 2000; Herkenham et al. 1990; Marsicano and Lutz 1999; Morales et al. 2007; Tsou et al. 1998) and receive inputs both from somatosensory (S1) and motor (M1) cortices (Alloway et al. 2006). The pontine nuclei (Pn), which contain CB1R expressing neurons (Cristino et al. 2006), receive inputs from CB1R regions of S1 (layer 5b; Mercier et al. 1990) and M1 (layer 5; Legg et al. 1989). Pn neurons project to the cerebellum (Cb), which also contain CB1R expressing neurons (Moldrich and Wenger 2000; Suarez et al. 2008; Tsou et al. 1998). Cerebellar Purkinje cells project to the inferior olive (IO), a CB1R-containing region (Suarez et al. 2008). Cb projections back to layer 3 of M1 via the ventral lateral thalamus and complete a cortico-cerebellar loop (VL; Yamamoto et al. 2004), and a sensory-motor loop via the IO, red nucleus (Rn) and SC (Suarez et al. 2008). CB1R has been proposed to be present in Rn (Tsou et al. 1998). The lateral facial nucleus (Fn) receive projections from a large number of regions, including M1 (Hattox et al. 2003), TN, and the deep gray layer of the SC (Miyashita and Mori 1995; Miyashita et al. 1994). The basal ganglia (BG), which express high amounts of CB1R, have not been included in this scheme because little is known about the involvement of this structure in whisking. The BG is mostly innervated by S1 pyramidal cells of CB1R expressing layer 5a (Mercier et al. 1990).

DISCUSSION

Dissociation between amplitude and frequency control in the vibrissal system

We found that Δ^9 -THC reduced net whisking spectral power, amplitudes, and peak angular velocities in a dose-dependent manner. In contrast, temporal parameters of whisking (whisking frequency, cycle duration, and bilateral synchrony) were marginally affected, with a significant effect only observed at the highest Δ^9 -THC dose (5 mg/kg). The CB1R antagonist, SR141716A, abolished all Δ^9 -THC-induced motor effects. By itself, SR141716A augmented whisker movement amplitudes, but did not affect whisking timing suggesting that SR141716A acts as an inverse agonist, reducing intrinsic CB1R activity or counteracting an endogenous cannabinoid tone involved in the modulation of whisking amplitude.

Whisking by control animals was centered at 7.0 Hz, with little frequency modulation (FM Δ) within bouts ($\Delta f_0 = 0.67$ Hz). The high spectral purity and the lack of Δ^9 -THC effect could suggest that cannabinoids and CB1Rs are not involved in FM. This absence of cannabinoid-mediated effects on FM is most likely not caused by frequency being inherently constant. Previous experiments showed that whisking frequencies of freely exploring rats vary between 5 and 20 Hz (Berg and Kleinfeld 2003). Modal whisking frequencies vary between unrestrained (9.3 Hz) and head restrained (6.1 Hz) rats whisking in free-air, although the spectral purity does not (Hill et al. 2008). Moreover, even in head-restrained rats, the whisking frequency is not constant and changes with deafferentation (Berg and Kleinfeld 2003; Gao et al. 2001b), motor cortex lesions (Gao et al. 2003), or tactile discrimination training (Harvey et al. 2001). The modal frequencies of head-restrained rats whisking in free air also differ between research groups, suggesting that small differences in experimental procedures can shift the dominant whisking frequency (Gao et al. 2001a; Hill et al. 2008; Knutsen et al. 2008). Thus whisking frequency could, in principle, be modulated under our experimental conditions. Indeed, we observed that after administration of a high Δ^9 -THC concentration (5 mg/kg), whisking frequencies were slightly reduced (1 Hz; Fig. 3B). However, no change in whisking frequency was observed during inhibition of endogenous cannabinoid signaling by the CB1R antagonist SR141716A. Thus we conclude that the endogenous cannabinoid system selectively modulates whisking amplitude, but not frequency, during exploratory whisking in air. During more complex behaviors when the head is moved and objects are being actively palpated, proprioceptive and sensory activation of sensory cortex may recruit endocannabinoid signaling (Ho et al. 2010) concomitant with changes in whisking frequencies. It remains possible that blocking or augmenting the effects of CB1R during such behaviors may result in timing related effects.

Our finding is consistent with anatomical and functional studies. Microstimulation, lesioning, electrophysiological, and pharmacological studies have shown that the reticular formation, and possibly motor cortex, are part of a network that regulates whisking frequency (Cramer and Keller 2006; Hattox et al. 2003), whereas the inferior olive, somatosensory cortex, and superior colliculus participate in the modulation of whisking amplitude (Gao et al. 2001b; Harvey et al. 2001; Hemelt and Keller 2008; Lang et al. 2006). Regions associated with

amplitude control express CB1R (Fig. 7B, green). In contrast, CB1Rs are largely absent in the sensory pathways of the vibrissal system (Fig. 7B, red). This segregation raises the possibility that whisking amplitude is controlled primarily by top-down motor channels belonging to high-order sensory-motor loops, whereas whisking timing is primarily regulated by lower-order sensory-motor channels.

Comparisons with other motor systems

DOSE-DEPENDENCY. Cannabinoids produce a range of motor effects (Navarro et al. 1993). Here, we report a monotonic, Δ^9 -THC dose-dependent reduction of whisking amplitudes. Other studies have described monotonic dose-dependent inhibition of locomotion and lever pressing by Δ^9 -THC (McLaughlin et al. 2000; Shi et al. 2005). On the other hand, at low doses, cannabinoids increase locomotion, whereas at higher doses, locomotion may be inhibited (Abel 1970a; Sulcova et al. 1998). Stimulatory effects were observed for other types of motor behaviors such as forepaw flutters, mouth movements, and scratching, Δ^9 -THC reduces motor activity at low doses, enhances it at higher doses, and inhibits movement at very high doses (Sanudo-Pena et al. 2000a).

SPECIFICITY. We observed a highly selective effect of Δ^9 -THC on whisking amplitude but not timing. Similar effects on amplitude versus timing have been reported by McLaughlin et al. (2000), showing that after Δ^9 -THC administration, peak force (amplitude) of lever pressing was reduced, but not the time to reach peak force.

Conclusions

Our primary finding reported here, that the endocannabinoid system is involved in selective modulation of whisking amplitudes but not timing, is consistent with anatomical and functional studies. It indicates that amplitude and timing can be controlled separately in the whisking system. Furthermore, it raises the possibility that whisking amplitude is controlled primarily by top-down motor channels, in which CB1Rs are expressed abundantly, whereas whisking timing is controlled primarily by sensory-motor channels, in which CB1Rs are expressed poorly. These results provide the first evidence of direct involvement of endocannabinoids in control of whisking and for separation of control functions between different channels of the whisking system.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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