

LACK OF RESPONSE SUPPRESSION FOLLOWS REPEATED VENTRAL TEGMENTAL CANNABINOID ADMINISTRATION: AN *IN VITRO* ELECTROPHYSIOLOGICAL STUDY

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Abstract—Cannabinoid compounds have been reported to excite ventral tegmental neurons through activation of cannabinoid CB1 receptors. More recently, biochemical and whole-cell voltage-clamp studies carried out on CB1-transfected AtT20 cells have shown a rapid desensitization of these receptors following activation of protein kinase C by 4- α -phorbol. To investigate the possible physiological correlates of this phenomenon, we have studied the effects of repeated cannabinoid treatment on ventral tegmental area dopaminergic neuronal firing *in vitro*. Rat brain slices containing the ventral tegmental area were used for single-unit extracellular recordings. Only neurons meeting established electrophysiological and pharmacological criteria for dopaminergic neurons were used in the study (firing neurons were detected either using tungsten or glass microelectrodes). The high-affinity cannabinoid agonist HU210 produced a concentration-dependent increase in firing (1–15 μ M; $EC_{50} \sim 7 \mu$ M). Initial HU210 exposure produced a significant increase in cell firing rate in the ventral tegmental area, with a maximum ~ 3.5 -fold increase over pre-drug basal firing; a subsequent exposure to HU210 produced an approximately threefold increase over basal firing. Nevertheless, the duration and onset of excitation produced by the cannabinoid differed significantly between the first and second exposures; the first excitation lasted significantly longer than the second and required less time to reach a comparable change in firing rate. The increases in firing rate and the time to return to basal firing were not significantly different between exposures. Furthermore, the cannabinoid antagonist SR141716A completely prevented the HU210-induced excitation whilst having no effect on its own, thus indicating a CB1-receptor mediated mechanism for the observed increase in firing. Ventral tegmental area neurons are also excited by the GABA_A receptor antagonist bicuculline. To assess the role of GABA in cannabinoid-mediated excitation, HU210 was added in the presence of bicuculline. HU210 did not affect the initial bicuculline-induced increase in firing, suggesting different sites of action for the two compounds.

Our data fail to support previously reported findings using repeated cannabinoid administration and cell preparations. The maintained increase in DA drive elicited by the potent cannabinoid agonist HU210 in the *in vitro* ventral tegmental circuit could explain some of the behavioural properties of cannabinoids, such as the lack of tolerance for the psychotropic effects of marijuana seen in human users. © 2000 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: ventral tegmental area, cannabinoids, DA, GABA, interneuron, reward.

The discovery of central cannabinoid receptors and associated endogenous ligands has facilitated the study of the pharmacology of marijuana. Previous studies have shown that the main psychoactive component of marijuana, Δ^9 -tetrahydrocannabinol, alters DA activity in the brain reward system originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens.¹¹ Accordingly, Δ^9 -tetrahydrocannabinol and other cannabinoids increase VTA DA neuron firing *in vivo* by a central cannabinoid (CB1) receptor-mediated mechanism.⁹ This activation leads to increased DA levels in terminal areas of the nucleus accumbens and the striatum measured by microdialysis.³³

However, a direct action of the cannabinoids on DA neurons in the VTA seems to be unlikely, as CB1 receptors are not localized on adult DA neurons.^{13,14,25} Thus, it is more plausible that the effects of cannabinoids occur via an alteration to afferent transmission within the VTA.¹⁷ One possible mechanism for cannabinoid-induced activation of VTA neurons could be inhibition of local interneurons that utilize

GABA as their neurotransmitter. A recent report by Katona *et al.*²⁰ showing that CB1 receptors regulate GABA release from presynaptic terminals of hippocampal interneurons supports this hypothesis. Nevertheless, autoradiographic and immunohistochemistry studies have shown that CB1 receptor levels are sparse within midbrain structures such as the VTA, possibly indicating a neuromodulatory role of the receptors.^{13,25} The latter is in agreement with 2-deoxyglucose autoradiographic studies that show areas in the brain where the cannabinoid receptor distribution does not match cannabinoid-induced changes in metabolism.²³

CB1 receptors belong to the G-protein (Gi)-coupled superfamily of seven-transmembrane spanning domain receptors.²⁴ Coupling of cannabinoid agonists to these receptors produces inhibition of adenylyl cyclase and modulation of several ion channels.³⁴ Recent biochemical and whole-cell voltage studies carried out in *Xenopus* oocytes and AtT20 cells transfected with CB1 receptors have shown a consequent phospholipase C stimulation (generating diacylglycerol and inositol triphosphate), leading to protein kinase C activation. Protein kinase C phosphorylation of the third intracellular loop of the receptor in turn disrupts cannabinoid-induced modulation of Kir and P/Q-type calcium channels in a rapidly desensitizing manner.¹⁰ Nevertheless, CB1 receptor internalization by a G-protein-coupled receptor kinase does not seem to play a role in the desensitization of the receptor, as the two processes can occur independently of each other.¹⁶

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Abbreviations: ACSF, artificial cerebrospinal fluid; DA, dopamine (3-hydroxytyramine); HU210, (–)-11-hydroxy- Δ^8 -tetrahydrocannabinol-dimethylheptyl; NMDA, N-methyl-D-aspartate; SR141716A, N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; VTA, ventral tegmental area.

To investigate the possible physiological and behavioural correlates of this rapidly developing tolerance, the present study determined the effects of a cannabinoid agonist and antagonist on the activity of DA neurons in the VTA and whether the effect of the agonist was altered with repeated administration. Furthermore, the study investigated whether the actions of the cannabinoid agonist involved GABAergic mechanisms.

EXPERIMENTAL PROCEDURES

Preparation of brain slices

Male Sprague–Dawley rats (250–300 g; Charles River, UK) were anaesthetized with halothane and decapitated using a guillotine; all experiments were performed under UK Home Office regulations (Project Licence PP 40/1955). The midbrain region was dissected immediately rostral to the cerebellum and caudal to the optic chiasma, then mounted on to a stainless steel cutting stage, which was placed in the specimen bath of a Vibroslice (Model 572, Campden Instruments, UK). The bath was filled with ice-cold oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid (ACSF; in mM: NaCl 124, KCl 3.3, KH₂PO₄ 1.2, MgSO₄ 1.0, CaCl₂ 2.5, NaHCO₃ 25.5 and D-glucose 10.0); coronal brain slices (400 µm thick) containing the VTA were cut and transferred to a 90-mm Petri dish filled with chilled and oxygenated ACSF. A small spatula was used to free the midbrain from overlying cortical structures in the section and the slice placed in a recording chamber.

Single-unit extracellular recordings from the ventral tegmental area slice preparation

The brain slice chamber consisted of a PDMI-2 open perfusion micro-incubator (Medical Systems Corp., USA) mounted on the stage of an Axiovert 100 inverted microscope (Zeiss, Germany), modified with the addition of an Olympus SZ trinocular stereo-zoom microscope (Olympus, Japan) to view the preparation. The VTA was visualized by trans-illumination as a semiluent area medial to the medial terminal nucleus of the accessory optic tract, an easily identifiable structure separating the VTA from the substantia nigra. The recording chamber consisted of a disposable tissue culture dish (35 mm in diameter) with the slice mechanically stabilized using a stainless steel insert that also reduced the volume of the chamber to 0.3 ml.⁵ The complete chamber was then placed into the micro-incubator central well and tissue was allowed at least 1 h equilibration time before starting any electrophysiological recordings. An LKB 2120 Variopex II peristaltic pump (LKB, Sweden) continually supplied pre-gassed and pre-warmed ACSF at a rate of 2.0 ml/min.

The temperature of the incubator was kept constant at 35°C with a TC-202 bipolar temperature controller (Medical Systems Corp., USA). Recordings were made with either glass micropipettes (pulled on a Narishige P-83 vertical puller; impedance of 18–20 MΩ) or tungsten electrodes (World Precision Instruments, USA; 5 MΩ). No difference was found in the type of recording obtained with either type of electrode. Electrode placement was achieved using a remotely controlled DC-3K motorized micromanipulator (MS314XYZ, Marzhauser, Germany) secured to the body of the microscope.

Neuronal spikes were amplified via a Neurolog a.c. preamplifier (NL104) and filtered (NL125, bandwidth 300 Hz–3 kHz) for subsequent display and discrimination on a Textronix 5000 series dual-beam oscilloscope; firing rate was also monitored aurally with the aid of a loudspeaker. Discriminated spikes were then collected through a Digidata 1200B interface on an IBM PC and plotted as integrated firing rate histograms over successive 5-s epochs using Axoscope (Axon Instruments, USA) and recorded in parallel on a chart recorder. All traces were saved on a CD-ROM and imported into Sigma Plot (SPSS, Rafael, CA, USA) for graphical representation.

Identification of DA neurons

Only neurons exhibiting characteristics of VTA DA neurons were studied.³⁶ These characteristics were long-duration (>2 ms) positive-negative action potentials and pacemaker-like firing rates of <5 spikes/s, in addition to being excited by *N*-methyl-D-aspartate (NMDA) and inhibited by DA.³⁵ No burst firing activity was observed

in any of the experiments. Neurons meeting these criteria were exposed to the potent cannabinoid agonist HU210, to the CB1 receptor antagonist SR141716A and to the GABA_A receptor antagonist bicuculline methobromide. All drugs were diluted in the superfusate to the desired concentration and their effects on the basal firing of VTA DA neurons monitored during and after 4-min perfusions. Only treatment-evoked responses showing a $\pm 20\%$ change from pre-drug basal firing were considered for analysis.

Materials

HU210 was obtained from Tocris Cookson (Bristol, UK), NMDA, DA and bicuculline methobromide were obtained from Sigma Chemical (Gillingham, UK). SR141716A was a generous gift from Sanofi Recherche. HU210 and SR141716A were resuspended to stock concentration (10⁻² M) in ethanol and frozen (–20°C) in 75-µl aliquots until required. The rest of the drugs were dissolved in distilled water. For each experiment an aliquot was thawed, diluted in ACSF to the desired working concentration, pH adjusted (if required), gassed, heated to 35°C and applied to the brain slice preparation through manual switching of the perfusion lines. The lag time for complete exchange following switching was achieved in 60–70 s and did not affect the firing rate of the neurons.

Data analysis

A total of 35 neurons (one neuron per animal) was assessed for their responses to the different drugs. Data are expressed as mean $\pm$ S.E.M. Statistical differences were determined using a one-way ANOVA (treatment $\times$ firing rate), followed by Tukey's post hoc test for comparison of firing rates across treatments. Paired Student's *t*-tests were used for single treatment experiments. The criterion of significance for the *t*-tests, the ANOVAs and post hoc tests was set at $P < 0.05$. All analyses were carried out with Prism statistical software (Graphpad, CA, USA).

RESULTS

Effect of HU210 on basal firing rate

A total of 20 neurons was assessed for their responses to the CB1 agonist HU210. In general, HU210 produced a concentration-dependent increase in basal firing (1.66 ± 0.9 Hz, $n = 20$), yielding a robust concentration–response curve ($3\text{--}15$ µM, $n = 5$; EC₅₀ ~ 8 µM; Fig. 1). Repeated administration of the cannabinoid agonist (up to five applications) produced a mild desensitization, i.e. less than a 20% change from the previous excitation (Fig. 2). Only the first two applications were used for statistical comparisons, as subsequent perfusions did not differ significantly from the first (data not shown). Both exposures to HU210 (5 µM) produced significant increases in cell firing, with a maximum fourfold increase over basal for the first administration and a threefold increase for the subsequent exposure. The duration (8.0 ± 1.1 and 6.0 ± 0.73 min, respectively; $P < 0.01$) and onset (0.9 ± 0.2 and 2.3 ± 0.7 min, respectively; $P < 0.05$) (Table 1) of the first excitation differed significantly from the second exposure. The first excitation lasted longer than the second and required less time to reach a comparable change in firing rate. The time to return to basal (recovery) (2.1 ± 0.21 and 2.0 ± 0.6 min, respectively) did not differ significantly between exposures (Table 1), nor did the overall increase in firing rate for all recorded cells (7.2 ± 1.4 and 6.6 ± 0.8 Hz, respectively).

Effect of SR141716A on DA neuron firing

HU210-evoked firing (at 10 µM) was markedly reduced by the cannabinoid receptor antagonist SR141716A at a concentration (1 µM, $n = 5$) that had no effect on pre-drug basal

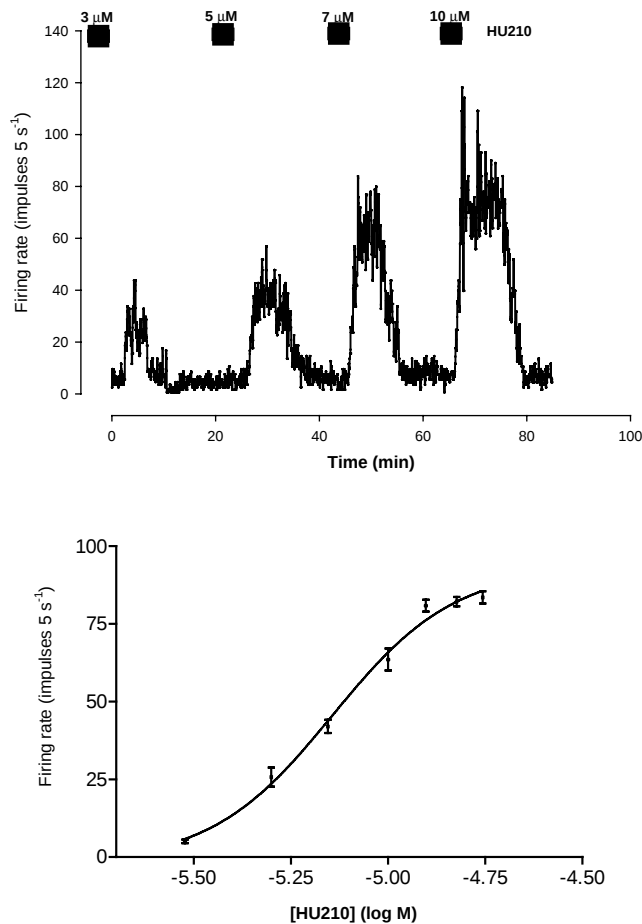


Fig. 1. (Upper panel) Representative integrated firing rate histogram (accumulated over successive 5-s epochs) of an identified VTA DA neuron illustrating the effects of increasing concentrations of the cannabinoid agonist HU210. This particular neuron responded in a concentration-dependent manner to the effects of HU210. The solid bars indicate the timing and duration of drug application (total $n = 5$). (Lower panel) Representative concentration–response curve for HU210-induced excitation of VTA neurons. The ordinate represents absolute firing rate as collected from the acquisition software.

firing (Fig. 3). Addition of the agonist in the presence of the cannabinoid antagonist (Fig. 3) completely abolished cannabinoid-induced firing. In contrast, when NMDA ($10 \mu\text{M}$) was added 10 min after the SR141716A and HU210 applications, the cannabinoid antagonist had no effect on the NMDA-induced increase in firing rate (data not shown).

Effect of bicuculline on ventral tegmental area DA neuron basal firing rate

Application of the GABA_A receptor antagonist bicuculline methobromide increased the firing rate of neurons in a concentration-dependent manner ($n = 5$; Fig. 4). Basal firing was increased approximately threefold, from 1.0 ± 1.8 to 3.9 ± 1.2 Hz ($10 \mu\text{M}$), and seven-fold, from 1.2 ± 3.6 to 9.3 ± 2.5 Hz ($20 \mu\text{M}$), respectively. Only the duration of the excitation differed significantly between the two doses of bicuculline (7.3 ± 1.2 and 12.5 ± 2.5 min for 10 and $20 \mu\text{M}$, respectively; $P < 0.05$) (Table 2). Addition of HU210 at a submaximal concentration ($10 \mu\text{M}$) in the

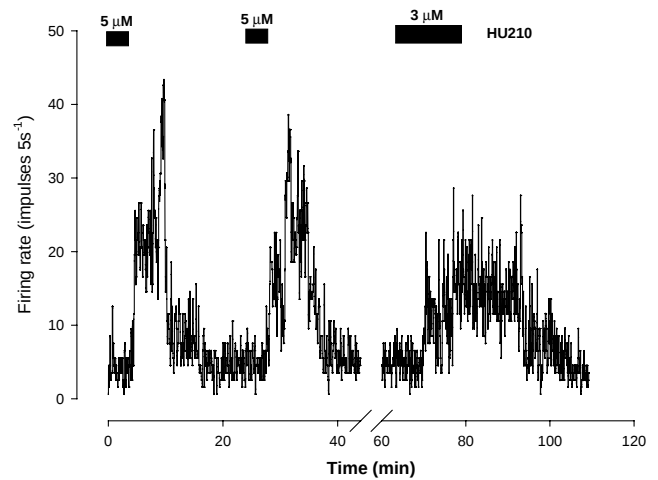


Fig. 2. Representative integrated firing rate histogram (accumulated over successive 5-s epochs) of an active VTA DA neuron during repeated 4-min applications of the cannabinoid agonist HU210 ($5 \mu\text{M}$; $n = 20$). This particular neuron showed no desensitization to the effects of HU210. When the cannabinoid agonist was applied at $3 \mu\text{M}$ for twice the duration of the previous applications (8 min), the neuron continued to discharge in a non-desensitizing manner throughout the perfusion. The solid bars indicate the timing and duration of drug application.

Table 1. Comparison of the cannabinoid agonist HU210-induced excitation duration, onset and recovery from the first and second HU210 applications

	Onset (min)	Excitation duration (min)	Recovery (min)
HU210 (1)	0.9 ± 0.2	8.0 ± 1.1	2.1 ± 0.21
HU210 (2)	$2.3 \pm 0.7^*$	$6.0 \pm 0.73^{**}$	2.0 ± 0.6

Data are mean $\pm$ S.E.M. ($n = 20$ cells for each measurement).

* $P < 0.05$, ** $P < 0.01$, compared with the first application.

presence of bicuculline had no effect on the bicuculline response (Fig. 5, Table 3).

DISCUSSION

Our results confirm a number of previous *in vivo* studies showing that cumulative dosing of various cannabinoids excites dopaminergic neurons in the VTA in a dose-dependent and CB1-specific manner.⁹ The present *in vitro* study shows that the ability of cannabinoids to excite VTA neurons is not altered by repeated administration. In our preparation, the cannabinoid agonist HU210 excited identified VTA DA neurons in a non-desensitizing manner, probably by reducing GABA inhibition of dopaminergic cells, in a way reminiscent of opiate action in the same brain area.¹⁷

Ventral tegmental area anatomical pathways and their relevance to cannabinoid-induced DA cell firing

The VTA contains a majority of DA ($\sim 70\%$) neurons and at least four subtypes of these neurons have been identified on the basis of their co-existence with one or more neuropeptides.^{7,15,21} The projection fields of these neurons appear to reach most densely limbic and cortical regions, especially the prefrontal cortex.³² Approximately 20% of the neurons in the VTA express mRNA for the GABA-synthesizing enzyme glutamate decarboxylase,

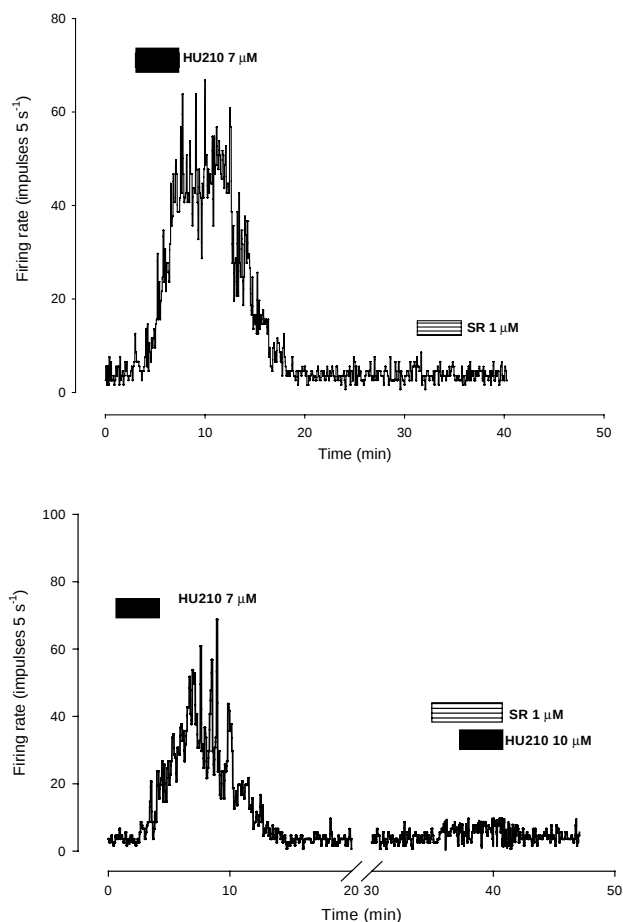


Fig. 3. (Upper panel) Representative integrated firing rate histogram depicting the effects of the cannabinoid antagonist SR141716A on an identified VTA DA neuron measured extracellularly. SR141716A ($1\text{ }\mu\text{M}$) did not affect the firing rate of identified neurons and reversed the cannabinoid agonist HU210 ($10\text{ }\mu\text{M}$)-induced excitation. (Lower panel) When added before HU210, SR141716A completely prevented the cannabinoid excitatory response. These recordings are representative of five identical experiments. The solid and hatched bars indicate the timing and duration of drug application.

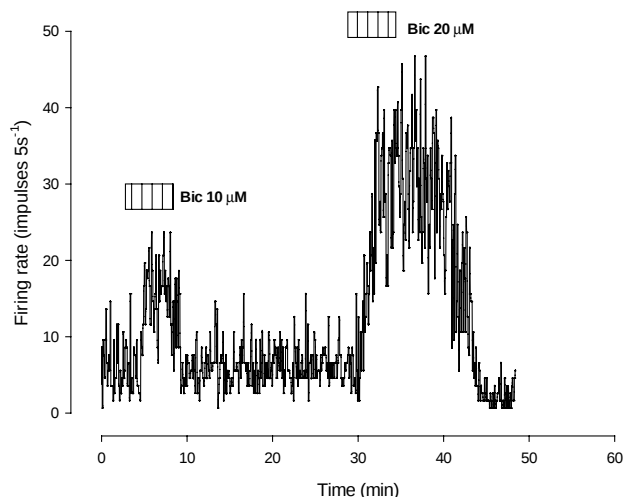


Fig. 4. Representative integrated firing rate histogram of an identified VTA DA neuron following two concentrations (10 and $20\text{ }\mu\text{M}$) of the GABA_A receptor antagonist bicuculline methobromide (Bic; total $n = 5$). Bicuculline, like the cannabinoid agonist HU210, produced a concentration-dependent increase in firing rate. The hatched bars indicate the timing and duration of drug application.

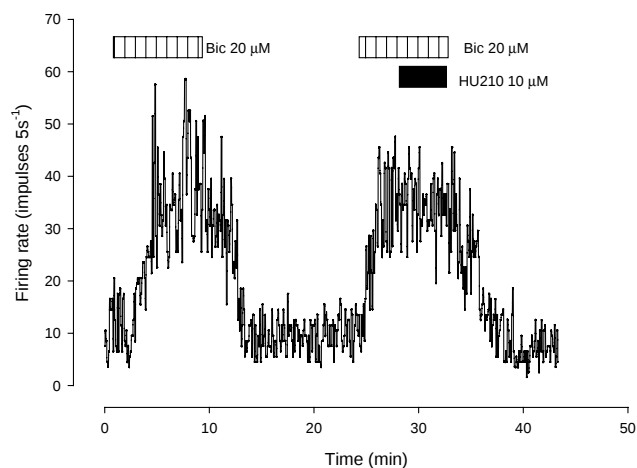


Fig. 5. Representative integrated firing rate histogram of an identified VTA DA neuron following bicuculline methobromide (Bic; $20\text{ }\mu\text{M}$); during the second bicuculline-induced response, the cannabinoid agonist HU210 ($10\text{ }\mu\text{M}$) was co-applied but was without effect on the initial plateau response ($n = 5$).

Table 2. Comparison of the duration of excitation, onset and recovery between the chosen bicuculline concentrations (10 and $20\text{ }\mu\text{M}$)

	Onset (min)	Excitation duration (min)	Recovery (min)
Bicuculline ($10\text{ }\mu\text{M}$)	1.12 ± 0.42	7.3 ± 1.2	2.03 ± 0.64
Bicuculline ($20\text{ }\mu\text{M}$)	1.6 ± 0.73	$12.5 \pm 2.5^*$	1.36 ± 0.56

Data are mean $\pm$ S.E.M. ($n = 5$).

* $P < 0.05$ compared with $10\text{ }\mu\text{M}$.

making GABAergic neurons the main source of DA neuron modulation within the tegmental circuit.^{20,31} Both GABA_A and GABA_B receptors are found in the VTA, although the density of these two receptors is low compared to the substantia nigra pars reticulata.¹⁸

All the neurons studied in this report complied with the previously established electrophysiological and pharmacological parameters used to identify mesolimbic DA neurons.^{1,3} The absence of burst firing in our slice preparation is also in agreement with the fact that the electrophysiological characteristics of these neurons are different when recorded *in vitro*.³⁶ These electrophysiological characterizations provide a critical but indirect approach to identifying DA neurons in the VTA involved in mesolimbic connectivity.

The finding that the firing rate of identified VTA DA neurons is modified by the repetitive addition of a CB1 receptor agonist to a brain slice containing the VTA indicates that the cannabinoids activate dopaminergic neurons located within the local VTA neuronal circuit in a non-desensitizing manner. The latter is in agreement with previous *in vivo* studies^{8,37} showing a lack of tolerance for cannabinoid-induced firing in VTA, but not substantia nigra pars compacta, neurons in chronically treated rats. The authors highlight a differential effect in terms of the development of tolerance for the euphorogenic (VTA-mediated) actions of the cannabinoids seen in human users of marijuana and other cannabinoid-mediated physiological effects (VTA independent). Although the present *in vitro*

Table 3. The onset, duration and recovery from the bicuculline-alone-induced excitation were not affected by the addition of HU210

	Onset (min)	Excitation duration (min)	Recovery (min)
Bicuculline (20 μ M)	1.81 $\pm$ 0.29	11.38 $\pm$ 0.83	1.41 $\pm$ 0.16
Bicuculline (20 μ M)/HU210 (10 μ M)	1.54 $\pm$ 0.37	12.27 $\pm$ 0.96	1.28 $\pm$ 0.31

Data are mean $\pm$ S.E.M. The animals used in this study were different from those used in the bicuculline-alone study ($n = 5$).

investigation supports the hypothesis that tolerance does not develop to VTA-mediated cannabinoid effects, it is conceded that the situation *in vivo*, given the disruption of regulatory neuronal networks in the slice preparation and its isolation from endocrine influences, might well be different.

Lack of effects of the cannabinoid antagonist SR141716A on the ventral tegmental circuit: absence of a mesolimbic cannabinoid tone?

The cannabinoid antagonist SR141716A had no effect on the basal firing of DA neurons, excluding the possibility of an endogenous cannabinoid tonic control over DA neurons within the VTA *in vitro*. SR141716A has also been reported to have no effect on VTA DA neuron firing *in vivo*.¹² However, further studies are required to exclude the possibility of an *in vivo* cannabinoid neuromodulatory tone, as the pharmacology of SR141716A is complex. Although SR141716A was first characterized as an antagonist at the CB1 receptor²⁷ (it antagonized established *in vivo* measures of cannabinoid activity collectively known as “the tetrad” in rodents), several membrane preparation studies from rat hepatocytes or CB2-transfected Chinese hamster ovary cells have shown that SR141716A poorly displaces cannabinoid ligands in these models.⁴ Furthermore, SR141716A has also been reported to be an inverse agonist in CB1 receptor-transfected Chinese hamster ovary cells and human tissue as assessed by guanosine-5'-O-(3-[³⁵S]thio)triphosphate binding.^{22,29} In addition, *in vivo* doses of the antagonist alone above 3 mg/kg stimulate locomotor activity and evoke significant thermal hyperalgesia in mice.²⁶ None the less, it is likely that the increase in DA neuronal activity elicited by cannabinoids in our preparation occurs via coupling and activation of CB1 receptors, as the addition of SR141716A prevented the HU210-induced increase in firing. Although these receptors exhibit very rapid internalization-independent desensitization in cell preparations,¹⁰ it appears that, in the presence of the intact local VTA neuronal circuit, this desensitization is prevented by afferents to the cells where the CB1 receptors are located.

Effect of GABAergic interneurons on DA neuron firing

Autoreceptor function and afferent input are critical in the understanding of the modulation of DA neuronal activity.³¹ To determine whether GABA was playing a role on the excitatory effects of the cannabinoids, we added HU210 in the presence of the GABA_A receptor antagonist bicuculline. The enhanced firing rate in the presence of bicuculline alone is consistent with the proposal that interneuronal GABA exerts a tonic control over DA neurons. The lack of an additive effect between the addition of bicuculline and the subsequent HU210 application suggests that the drugs have a common

cellular target and that the cannabinoid agonist might act via the inhibition of interneuronal GABA release. The latter lends support to the notion of a different location of the two receptors, as GABA_A receptors are primarily postsynaptic whereas CB1 receptors are located mainly presynaptically and do not seem to be present on adult dopaminergic cells.¹⁸ Interestingly, Hernández *et al.*¹⁴ have recently shown that CB1 receptors co-localize with tyrosine hydroxylase-positive neurons in fetal mesencephalic cultures, suggesting a direct action of endogenous cannabinoids on DA neurons during brain development.

Immunohistochemical studies have shown that the distribution of CB1 receptors in the hippocampus is mainly presynaptic on nerve terminals of GABAergic interneurons (cholecystokinin-containing basket cells).²⁰ These receptors control the release of GABA and therefore modulate hippocampal network activity. Furthermore, intracellular recordings have shown that bath application of cannabinoids on to identified GABAergic neurons significantly reduces the amplitude of nigraly evoked inhibitory postsynaptic currents.² This reduction results from a presynaptic mechanism in which the release of GABA is prevented by the cannabinoids, as GABA-induced currents are unaffected by the presence of the cannabinoid agonist. Taken together, these two factors lend support to the hypothesis that the presence of CB1 receptors located on the terminals of GABAergic interneurons in the ventral tegmentum may play a pivotal role in the excitatory effects of the cannabinoids, by inhibiting GABA inhibition of DA neurons in the VTA. These findings provide further evidence that GABA inhibition of DA neuronal function may be an important regulatory factor in the control of reward behaviour relevant to the actions of cannabinoids.

Ventral tegmental area DA neuron activity and its relevance to cannabinoid-mediated reward

Studies on cannabinoids produce conflicting results when behavioural paradigms are used to assess reward.¹¹ In addition, increases in VTA DA neuronal firing rate can be brought about by a stressor, a novel stimulus or a conditioned stimulus that predicts reward.¹⁸ Moreover, lesions of DA innervation have shown that the effects of DA originating in the VTA take place in the terminal nuclei, where neurotransmitter release occurs.^{19,30} These terminal areas include the nucleus accumbens but also the prefrontal cortex, an area linked to stress pathways.⁶

Increases in VTA dopaminergic cell firing elicited by the cannabinoid, as seen in our *in vitro* preparation, could therefore be brought about *in vivo* regardless of the motivational properties of the compound. Thus, DA can be regarded as a novelty predictor subserving a gating role in the effects that it mediates.¹⁸ Alternatively, DA-independent reward

mechanisms may play a role in the motivational characteristics of abused drugs.³¹ The latter has recently been highlighted by the self-administration of cocaine in DA-transporter knockout mice.²⁸

CONCLUSIONS

The results presented here show that a potent cannabinoid agonist repetitively stimulates the local ventral tegmental

circuit locally by a CB1 receptor-mediated mechanism. The excitations caused by the cannabinoids apparently result from inhibition of local circuit GABAergic interneurons that ultimately leads to a partially desensitizing disinhibition of DA cell firing. The relevance of ventral tegmental DA cell firing in terms of the rewarding properties of marijuana remains to be determined in light of the results obtained with animal models of drug dependence.

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