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Cocaine must enter the brain to evoke unconditioned dopamine release within the nucleus accumbens shell

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ABSTRACT

In addition to blocking dopamine (DA) uptake, cocaine also causes an unconditioned increase in DA release. In drug naive rats, this effect is most robust within the nucleus accumbens (NAc) shell. Recent studies have shown that, in rats trained to self-administer cocaine, cocaine may act in the periphery to enhance mesolimbic DA release. Further, these studies have suggested that peripheral cocaine action may also enhance unconditioned DA release. Here, we test if it is necessary for cocaine to enter the brain to evoke unconditioned increases in DA release within the NAc shell. Administration of a cocaine analogue that crosses the blood brain barrier (cocaine HCl) enhances electrically evoked DA release and the number of cocaine-evoked phasic DA release events (i.e., DA transients) within the NAc shell. However, administration of a cocaine analogue that does not cross the blood brain barrier (cocaine MI) does not alter either measure. We therefore conclude that cocaine must act within the central nervous system to evoke unconditioned DA release within the NAc shell.

Cocaine significantly impacts reward and motivation through increasing dopamine (DA) neurotransmission [10,19,37,50]. It is well established that cocaine initially increases extracellular DA concentration through inhibition of DA transporters (DAT) [15,24,42]. However, recent studies using fast-scan cyclic voltammetry (FSCV) in freely moving rats have shown that, in addition, cocaine enhances DA release by increasing the magnitude of DA release events (as demonstrated using electrical stimulation of dopaminergic afferents) [11,22,27,47] and by increasing the frequency of DA 'transients' [1,2,8,41]. A DA transient is defined as a phasic surge in DA concentration (5-fold increase in signal to noise) that lasts ~1 s [35,36,44,49]. These events are detected only in awake (freely moving) subjects (but can be pharmacologically evoked in anesthetized rats [30,48]). They do not appear to be caused by any obvious environmental stimulation, and, therefore, they are often referred to as 'naturally occurring' or 'spontaneous' phasic release events [1,8,41,49].

Cocaine-evoked increases in striatal DA concentration are greatest within the nucleus accumbens (NAc) shell [1,33] and cocaine-evoked increases in the frequency of DA transients are much greater within the NAc shell compared to the NAc core [1,2]. Moreover, blockade of DA neuron activity within the ventral tegmental area (VTA) prior to intravenous cocaine delivery eliminates cocaine-evoked increases in DA transients and elevated DA concentration within the NAc shell [1,41]. Therefore, this shell-selective increase in DA transients is responsible for the preferentially high levels of unconditioned increases in DA concentration within the NAc shell following cocaine delivery in animals with little to no experience with the drug [1,26,33]. Given that DA transmission within the NAc shell is important for enhancing drug reward and motivation [9,13,18], the current study was conducted to better understand the mechanism underlying unconditioned cocaine-evoked DA release within this region by measuring the number of DA transients (to approximate the contribution of phasic DA release events) as well as the level of DA concentration (to approximate the overall increase in DA transmission resulting from the sum of all mechanisms that enhance extracellular DA levels). While the robust increase in DA transients within the NAc shell following cocaine enhances the accumulation of DA in the extracellular space, there are certainly other mechanisms that contribute to this increase in concentration (i.e., uptake inhibition; see [1] for more discussion on this topic).

Conditioned stimuli that predict cocaine delivery also increase DA release [2,32,44,51] and it has recently been reported that, in rats trained to self-administer cocaine, the interoceptive cues associated with a cocaine injection increase glutamate transmission within the VTA, and thereby likely increases DA release [52,53]. Moreover, it was suggested this effect is caused by peripheral

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actions of cocaine because administration of cocaine methiodide (MI; a quaternary cocaine salt that does not cross the blood brain barrier; BBB) also increased intra-VTA glutamate [52]. Further, it has now recently been suggested that the modest unconditioned increases in VTA glutamate and therefore putative increase in DA neuron firing are also due to peripheral actions of cocaine [52] and that such peripheral effects may even contribute to experimenter administered electrical stimulation [51]. It is therefore important to test if peripheral actions of cocaine contribute to the unconditioned DA release evoked by this drug. Additionally, while previous studies have demonstrated that central mechanisms contribute to cocaine-evoked increases in DA release [12,27], the present study also determines if peripheral mechanisms also contribute to this effect. Here, we used FSCV in freely moving rats to determine if cocaine must enter the brain to increase DA release in drug naive subjects. Specifically, we tested if a cocaine analogue that is able to cross the BBB (cocaine HCl) differs from the analogue that does not enter the brain (cocaine MI) with respect to enhancing electrically evoked DA release as well as cocaine-evoked increases in DA concentration within the NAc shell. We report that cocaine must enter the brain to increase unconditioned DA release within the NAc shell.

Male Sprague-Dawley rats were purchased with surgically implanted intrajugular catheters ($n = 9$; 280–320 g; Charles River Laboratories) and housed in a 12 h reversed light–dark cycle with *ad libitum* access to food and water. Surgeries were conducted as previously described [1,2,8]. Briefly, animals were anesthetized with an intramuscular injection of a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). A guide cannula (Bioanalytical Systems) was placed over the NAc shell (AP: +1.8, ML: +0.8 from bregma) and a Ag/AgCl reference electrode was implanted into contralateral cortex (AP: −0.8, ML: −4.0 from bregma). A bipolar stimulating electrode (Plastics One) was positioned over the VTA (AP: −5.2, ML: +1.0 from bregma) and lowered to 7.0 mm from the brain surface. Stimulations (60 Hz, 60 pulses, 120 μA) were delivered as the electrode was lowered in 0.2 mm increments until an optimal level of stimulated DA was achieved (typically 8.6 mm below the brain surface). Rats recovered from surgery for 5–7 days, and on the experimental day a fresh carbon-fiber electrode was used for FSCV measures. Upon completion of the experiment, animals were euthanized with an overdose of intravenous (i.v.) ketamine, an electrolytic lesion was made at the voltammetric recording site, and electrode placement was verified histologically. All experiments were conducted under the approval of the University Committee on Use and Care of Animals of the University of Michigan. Cocaine HCl and cocaine MI were provided by NIDA.

To determine whether central cocaine action was required to enhance electrically evoked DA release in freely moving rats ($n = 4$), a fresh carbon-fiber microelectrode was lowered into the NAc shell into a location that supported electrically stimulated DA release [32,38]. To ensure that electrically stimulated DA release was stable, we delivered 9 electrical stimulations of the VTA (24 pulses, 60 Hz, 120 μA) with an inter-stimulation interval of 2.5 min. Previous studies have demonstrated that a properly functioning carbon-fiber electrode will record quite stable DA release concentration with long inter-stimulation intervals such as 2.5 min. Here, data were considered stable if the magnitude of stimulation-evoked DA release did not vary more than 20%. If variation in electrically evoked DA release was greater than 20%, the electrode was regarded as unfit for the experiment and was replaced. Data from this 'baseline' condition were identical to the 'saline' condition and are therefore not shown.

To control for the effects of i.v. vehicle infusion, we assessed stimulated DA release following a control i.v. infusion of saline (0.2 ml over 6 s). Three stimulations were delivered prior to an infusion, and the first 'post-infusion' stimulation was given 1 min after i.v. saline, and five additional stimulations followed at 2.5 min intervals. The precise times of electrical stimulations relative to i.v. infusions were: −6.5, −4.0, −1.5, 1.0, 3.5, 6.0, 8.5, 11.0 and 13.5 min. Fifteen minutes after the last stimulation in the saline condition, the same series of stimulations were delivered following either an i.v. infusion of cocaine HCl, at a dose that is self-administered (1.0 mg/kg) or a slightly higher dose of cocaine MI (1.3 mg/kg). This dose of cocaine MI was chosen because is has a weaker affinity for DA transporters compared to cocaine HCl [16] and previous studies have suggested that these are equimolar doses [12,52]. Since subjects received both cocaine HCl and cocaine MI, drugs were given in counter-balanced fashion and there was no effect of drug order. Thus, 45 min after the first drug injection, the second cocaine analogue was infused and electrical stimulations were delivered yet again. The stimulations prior to i.v. infusions across conditions were compared to determine electrode stability over the course of the experiment. To control for variability in the magnitude of electrically evoked DA release (largely due to placement of the stimulating electrode) DA release magnitudes were normalized to the average value of the 3 stimulations that immediately preceded i.v. infusion for each condition.

Similar to previous studies [4,23,28,31], multiple electrical stimulations with long time intervals between stimulations yielded very stable peak amplitudes across stimulated DA release events (Fig. 1). Control saline infusions had no effect on the magnitude of electrically evoked DA release (Fig. 1A and D). Following an i.v. infusion of cocaine HCl (1 mg/kg), peak DA concentration following electrical stimulation significantly increased 1 min ($F(1,3) = 17.63$, $p < .05$) and 3.5 min ($F(1,3) = 20.69$, $p < .05$) after drug infusion but not thereafter, and mean stimulated DA release values returned to baseline levels by 13.5 min after drug infusion (Fig. 1B and E). In contrast, cocaine MI had no effect on stimulated DA release (Fig. 1C and F). These data demonstrate that cocaine must enter the brain for it to enhance the magnitude of DA release by electrical stimulation of DA afferents. It must be noted that while we refer to electrically evoked increases in DA concentration as 'release' because it is release dominated, this measure is also influenced by DA uptake [14].

Previous studies have shown that i.v. cocaine administration causes a robust and unconditioned increase in DA concentration within the NAc shell that is due, at least in part, to an increase in the frequency of DA transients [1,2,41]. Therefore, we determined if cocaine entry into the brain is necessary for cocaine to increases DA concentration and to increase the frequency of DA transients within the NAc shell, using a separate group of subjects ($n = 5$). Carbon-fiber electrodes were lowered into the NAc shell and subjects first received a control infusion of saline (0.2 ml infused in 6 s). A representative DA concentration trace following saline infusion shows that this injection did not alter DA transmission (Fig. 1G). Infusion of cocaine HCl (Fig. 1H), but not cocaine MI (Fig. 1I) evoked many DA transients. Quantification of DA transients shows that cocaine HCl significantly increased the number of DA transients following the infusion, relative to transient frequency following saline or cocaine MI infusions (Fig. 1J; $F(1,4) = 13.78$, $p < .05$). Regarding overall DA concentration levels, control saline infusions did not alter DA transmission (Fig. 1K; $t(4) = 0.268$, $p > .05$). As expected, infusion of cocaine HCl increased DA concentration within the NAc shell (Fig. 1K; $t(4) = 2.86$, $p < .05$). However, infusion of cocaine MI did not increase DA concentration (Fig. 1K; $t(4) = -0.05$, $p > .05$). These data demonstrate that cocaine must enter the brain to cause an unconditioned increase in DA concentration within the NAc shell that is driven by an increase in the number of phasic DA release events.

The classic view of cocaine action is that it increases DA levels solely by slowing DA uptake, thus allowing DA to accumulate in the extracellular volume [15,24]. Although inhibition of catecholamine uptake is a major mechanism of cocaine action [34,46], there is now