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AVERSIVE STIMULUS DIFFERENTIALLY TRIGGERS SUBSECOND DOPAMINE RELEASE IN REWARD REGIONS

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Abstract—Aversive stimuli have a powerful impact on behavior and are considered to be the opposite valence of pleasure. Recent studies have determined some populations of ventral tegmental area (VTA) dopaminergic neurons are activated by several types of aversive stimuli, whereas other distinct populations are either inhibited or unresponsive. However, it is not clear where these aversion-responsive neurons project, and whether alterations in their activity translate into dopamine release in the terminal field. Here we show unequivocally that the neurochemical and anatomical substrates responsible for the perception and processing of pleasurable stimuli within the striatum are also activated by tail pinch, a classical painful and aversive stimulus. Dopamine release is triggered in the dorsal striatum and nucleus accumbens (NAc) core by tail pinch and is time locked to the duration of the stimulus, indicating that the dorsal striatum and NAc core are neural substrates, which are involved in the perception of aversive stimuli. However, dopamine is released in the NAc shell only when tail pinch is removed, indicating that the alleviation of aversive condition could be perceived as a rewarding event.

Key words: voltammetry, tail pinch, nucleus accumbens.

Dopamine signaling in the nucleus accumbens (NAc) is involved in the integration of sensory information and the initiation of the subsequent behavioral responses to diverse stimuli (Leknes and Tracey, 2008). The perception and behavioral consequences of rewarding and aversive stimuli are extremely different, and the underlying neural substrates mediating these opposing phenomena are unclear. Increased subsecond dopamine release in this brain region has been demonstrated with the presentation, seeking, and anticipation of reward (Phillips et al., 2003; Day et al., 2007; Roitman et al., 2008) as well as learning of reward-related information (Day et al., 2007; Owesson-White et al., 2008; Roitman et al., 2008). According to the reward coding hypothesis (Schultz, 1998; Montague et al., 2004a; Ungless, 2004; Ungless et al., 2004), mesolimbic dopamine neurons are inhibited or unresponsive to aversive stimuli, and increased dopamine release only occurs with reward-related stimuli, for example, the inhibition of dopamine release in rat NAc was observed during a 4-s intraoral exposure to quinine in a taste aversion model (Roitman et al., 2008). Others have suggested an alternative hypothesis in which increases in phasic dopamine activity can be initiated by any salient stimuli regardless of valence, including aversive stimuli (Redgrave et al., 1999, 2008; Anstrom et al., 2009). In support of this claim, it has been shown that significant increases in subsecond dopamine transmission occur in the NAc of rats under the aversive condition of social defeat stress (Anstrom et al., 2009).

Recently, an electrophysiology study discovered regional differences in dopamine neuronal firing within the ventral tegmental area (VTA) of anesthetized rats exposed to brief (4 s) foot shock (Brischoux et al., 2009). They found that foot shock inhibited dopamine neurons in the dorsal VTA, whereas those in the ventral VTA were phasically excited. The study performed on behaving mice showed that the majority of the VTA dopamine neurons exhibit decreased activity, although a small group of dopaminergic-like neurons can be activated by fearful events (Wang and Tsien, 2011). Another study in mice reported nearly equivalent proportions of dopamine neurons activated, inhibited, or unaltered by 5-s tail pinch (Zweifel et al., 2011). Therefore, electrophysiological studies clearly demonstrate that the activity of a subset of dopamine neurons in the VTA and substantia nigra can increase in response to aversive stimuli (Zweifel et al., 2011; Brischoux et al., 2009; Matsumoto and Hikosaka, 2009; Wang and Tsien, 2011). However, it is still not known how prevalent these aversion-excitable dopaminergic neurons are, and whether their increased activity translates into dopamine release in the terminal fields. Notably, changes in the activity of dopaminergic neurons in the VTA do not necessarily produce a proportional response in dopamine release in the terminal regions because terminal mechanisms and conditions may also shape the temporal profile of extracellular dopamine (Montague et al., 2004b; Wightman et al., 2007; Lammel et al., 2008). To address the fundamental question of whether and how dopamine neurotransmission within the NAc encodes aversive stimuli, we have assessed dopamine dynamics in real time in response to the classical aversive stimulus, a 3-s tail pinch.

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Abbreviations: FSCV, fast-scan cyclic voltammetry; NAc, nucleus accumbens; VTA, ventral tegmental area.

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EXPERIMENTAL PROCEDURES

Fast-scan cyclic voltammetry (FSCV)

Naive male Sprague–Dawley rats weighing approximately 350 g were housed with *ad libitum* food and water in a 12/12-h light/dark cycle. All procedures were approved by the Wake Forest University and University of North Carolina Animal Care and Use Committees. The study was designed to minimize the number of animals used and pain related to experimental procedures. Experiments were performed on anesthetized animals. Naive rats were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame. A carbon fiber electrode (50–100 μm exposed tip length, 7 μm diameter, Goodfellow, Oakdale, PA, USA) was positioned in the NAc core (AP +1.3, L +1.3, V −6.3 to −7.2 mm from bregma), or NAc shell (AP +1.8, L +0.9, V −6.8 to −7.2 mm), or dorsal striatum (AP +1.3, L +1.3, V − to −5.0 mm) (three separate groups of rats, n=5−6 per group). An Ag/AgCl reference electrode was inserted in the contralateral hemisphere. The reference and carbon fiber electrodes were connected to a voltammetric amplifier, and voltammetric recordings were made at the carbon fiber electrode by applying a triangular waveform (−0.4 to +1.3 V vs. Ag/AgCl, 400 V/s).

To avoid tissue damage and electrical noise artifacts during voltammetric recordings, the tail pinch was performed with soft rubber gloves. The tail of the rat was placed between the thumb and the index finger and pinched for a period of 3 s. The pinch was administered 1 cm from posterior tip of tail with maximal pressure (P) of 3.12±0.62 MPa. P was calculated by measuring the contact area between the fingers and the tail and by a measurement of the applied force using a Pasco CI-6537 Force Sensor (Roseville, CA, USA). This test was repeated four times with intervals of 2–3 min between each pinch. No reactions such as ear or whisker twitches were observed during this procedure. At the end of experiment, GBR 12909 (15 mg/kg, i.p.), a selective dopamine transporter inhibitor, was injected for the pharmacological confirmation of the pinch-induced electrochemical signal.

Data were digitized and stored on a computer. Carbon fiber electrodes were calibrated *in vitro* with known concentrations of dopamine (0.2, 0.5, and 1.0 μM). Calibrations were done in duplicate, and the average value for the current at the peak oxidation potential was used to normalize *in vivo* signals to dopamine concentration. When the carbon fiber electrode was used to lesion the brain for placement verification, an averaged post calibration was employed to calculate dopamine concentration (Park et al., 2010).

Chemicals and drugs

All chemicals and drugs were reagent quality and were used without additional purification. Drugs were obtained from Sigma-Aldrich (St. Louis, MO, USA). *In vitro* dopamine calibration of microelectrodes was performed in a tris buffer solution at pH 7.4 containing 15 mM Tris, 140 mM NaCl, 3.25 mM KCl, 1.2 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 1.2 mM $MgCl_2$, and 2.0 mM Na_2SO_4 in double distilled water (Mega Pure System, Corning Glasswork, Corning, NY, USA). GBR 12909-HCl was dissolved in a small volume of distilled water and diluted with saline. Injected volumes were 1 ml/kg and were given i.p.

Histological verification of electrode placement

Electrode placements were verified stereotaxically as described previously (Anstrom et al., 2009; Park et al., 2010). A lesion was made at the recording site by applying constant current (20 μA for 10 s) to the tungsten or carbon fiber electrodes. Brains were removed from the skulls and stored in 10% formaldehyde for at least 3 days, then they were coronally sectioned into 40–50-μm-thick slices with a cryostat. The sections mounted on slides were stained with 0.2% Thionine and coverslipped before viewing under a light microscope. Fig. 5 demonstrates anatomical distribution of carbon fiber electrode placements in the NAc shell, NAc core, and dorsal striatum. Coronal diagrams show electrode tip locations for 18 rats. Coordinates were taken from a stereotaxic atlas.

Statistical analysis

Data were analyzed in GraphPad Prism (GraphPad Software, San Diego, CA, USA). Paired *t*-test and 1- and 2-way ANOVAs with a Bonferroni post tests were used to determine statistical significance. The data are presented as mean±SEM, and the criterion of significance was set at $P<0.05$.

RESULTS

A tail pinch induced a significant increase in extracellular dopamine concentration in the rat striatum (Fig. 1). However, significant differences in tail pinch-evoked dopamine release were revealed between striatal subregions (1-way ANOVA; $F[2,15]=10.75$, $P<0.005$). Specifically, dopamine release in the dorsal striatum was significantly less than in the NAc core and shell (Bonferroni's multiple comparison test, $P<0.01$, n=6 per group, Fig. 2A). No difference was observed between subregions of NAc ($P>0.05$). Importantly, there was a significant difference in the onset of pinch-induced dopamine release between NAc core and shell (1-way ANOVA; $F[2,15]=66.89$, $P<0.0001$). Dopamine release in the NAc shell was notably delayed in comparison with that in the dorsal striatum and NAc core (Bonferroni's multiple comparison test, $P<0.001$, n=6 per group). At the same time, dopamine release in the NAc core and dorsal striatum was closely coupled to the stimulus (Fig. 2B). Importantly, tail pinch induced a stable response in all tested striatal subregions (Fig. 3). There was no significant difference in the amplitude of dopamine efflux after four sequential tail pinches (2-way ANOVA; $F[3,48]=0.1909$, $P>0.05$, n=5 per group). Dopamine responses, which were detected in the dorsal striatum, NAc core, and NAc shell after the first pinch were 21.3±2.7, 36.4±10.1, and 48.9±12.3 nM, respectively (Fig. 3). We also assessed dopamine release changes in the NAc core in response to a foot pinch. The amplitude of dopamine efflux, which was reached during the 3-s stimuli, did not