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Supplemental Data

Cocaine but Not Natural Reward Self-Administration nor Passive Cocaine Injection Produces Persistent LTP in the VTA

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Food schedule

After recovery from catheter or sham surgeries (7 days), all animals were food deprived for 24 hrs prior to the overnight session. During the course of training, food intake was increased gradually and similarly in all groups, from 18g/day to ad lib access to food by the 7-9th day of operant training (Cocaine, Food) or exposure to the operant chamber (Yoked). Throughout the remainder of training as well as during the 21 days of abstinence, all animals received ad libitum home cage access to food and water. Animals from all groups exhibited similar rate of weight gain throughout experimentation.

Aged-match naïve rats (Naïve)

12 Naïve rats were used. Rats in this group arrived at the same time as all the other groups of animals. 5 rats were sacrificed at the same time as the 1 day group and 7 rats were sacrificed at the same time as the 21 days abstinence group. No differences in AMPAR/NMDAR and mEPSC frequency or amplitudes were observed between the two sets of Naïve rats and data were pooled.

Cocaine self-administration rats

Lever pressing in Cocaine rats was initially shaped in one or two (depending on whether animals acquired the operant behavior) 15-hr, overnight sessions following a 24-hr food deprivation. If a second overnight session was required, animals were food restricted to 15g of food in between the two overnight sessions. During the overnight session(s), depression of the active lever on a fixed ratio (FR-1) resulted in the delivery of a food pellet (45mg, Noyes, Lancaster, NH) and illumination of a cue light directly over the

active lever for 4 sec. Pellet delivery was followed by an 18-sec timeout period where responses were recorded, but resulted in no consequence. After lever pressing was acquired during the overnight session(s), animals were placed for one day on a conjoint (same lever) 2-hr FR-1 food, FR-5 cocaine schedule (cocaine from Sigma; 0.25 mg/kg/infusion over 2 sec). The cue light above the active lever was illuminated for 4 sec upon completion of the FR-1 food schedule. Upon completion of the FR-5 cocaine schedule, both the cue light above the active lever was illuminated and a tone sounded for 4 sec, and both cocaine (0.25 mg/kg over 2 sec) and a food pellet were delivered. All reinforcers (food and/or cocaine) were followed by an 18-sec timeout in which responses were recorded but had no consequence. The session with both food and cocaine as reinforcers facilitated the transition to cocaine-only self-administration. The next day, training continued for cocaine on an FR-1 schedule (0.25 mg/kg/infusion over 2 secs, followed by an 18-sec timeout), 2 hrs per day. Food pellets were no longer delivered but the light and the tone were activated for 4 sec upon lever pressing. The house light was illuminated throughout each session.

Rats were trained for at least 14 days, after which training continued until reaching the maintenance criteria of less than 15% variability in responding on the active lever over three consecutive days. Rats that did not reach this criterion in 14 days were trained until the criterion was met.

Catheter patency for Cocaine and Yoked groups was tested at the end of the behavior training with brexital. Animals were given 0.3ml brexital (3 mg/ml; Brexital Sodium, Monarch Pharmaceutical, Bristol, TN) via the jugular catheter.

Food and sucrose self-administration rats

Lever pressing in food rats was initially shaped in one or two (depending on whether animals acquired the operant behavior) 15-hr, overnight sessions. If a second overnight session was required, animals were food restricted to 15g of food in between the two overnight sessions. During the overnight session(s), depression of the active lever resulted in the delivery of a food (45mg pellet, Noyes) or sucrose (45mg pellet, Test Diet, Richmond, IN) pellet and illumination of a cue light directly over the active lever for 4 sec. The next day, training continued for food or sucrose pellets on an FR-1 schedule, 2

hrs per day. Depression of the active lever resulted in the delivery of a food or sucrose pellet, illumination of a stimulus light over the active lever, and a 4 sec tone. Reinforcer delivery was followed by an 18-sec timeout. Lever presses were recorded during the timeout, but resulted in no consequence. As with Cocaine rats, training continued until the same criteria were reached (less than 15% variability in responding on the active lever over three consecutive days) and for at least 14 days. At this point, Food and Sucrose rats were divided into behaviorally-matched groups according to their operant responding. The first represented the one-day abstinent group, the second group was used for the 7-days abstinent group, and the third group was used for the 21-days abstinent group. All rats assigned to the Food and Sucrose group were successfully trained to criterion.

Yoked Rats

Yoked rats experienced a 15 hr, overnight session in the operant chamber. During the overnight session, both levers were extended but were inactive, and presses on the levers had no programmed consequences. The next day, Yoked rats were placed in the operant chamber for 2 hrs, where they received cocaine non-contingently through a computer-generated yoked design. The yoking program was developed because yoking animals 1:1 with Cocaine self-administering animals using the Coulbourn software monopolizes equipment (where only one Cocaine animal can be yoked to any number of controls). To generate the yoking protocol, a series of inter-infusion intervals was derived from the response patterns of Cocaine animals. Because cocaine self-administration increased with training in the Cocaine group, four series were generated that corresponded to the response patterns of Cocaine animals during the 1st-4th, 5th-8th, 9th-12th, and 13th-19th days of training.

During a 2 hr yoked session, the computer randomly selected an inter-infusion interval with replacement from the series appropriate to the time point during training. The computer waited for the duration of that inter-infusion interval prior to executing a probability function, where an animal was infused with 0.25 mg/kg or 0.50 mg/kg cocaine infusion over 2 sec either 70 % of the time (during the 1st-8th day of training) or 85% of the time (during the 9th-18th day of training). Different probability functions were used at different times during training so that Yoked infusions closely resembled the

increasing cocaine intake observed in the Cocaine group over time. Thus, this procedure ensured that individual animals experienced a truly random yoke pattern over days as well as between animals within a single day that, nonetheless, closely resembled the operant population. In addition, cocaine infusion in Yoked animals was not paired with light or tone. Thus, Yoked rats never associated discrete stimuli or operant responding with cocaine and therefore served as controls for cocaine self-administration.

Roughly 15% of Yoked animals were removed from the study due to catheter failures. Yoked animals received non-contingent cocaine infusions for at least 14 days.

Supplemental Table 1. Summary of behavior responses from Cocaine, Food, and Sucrose groups.

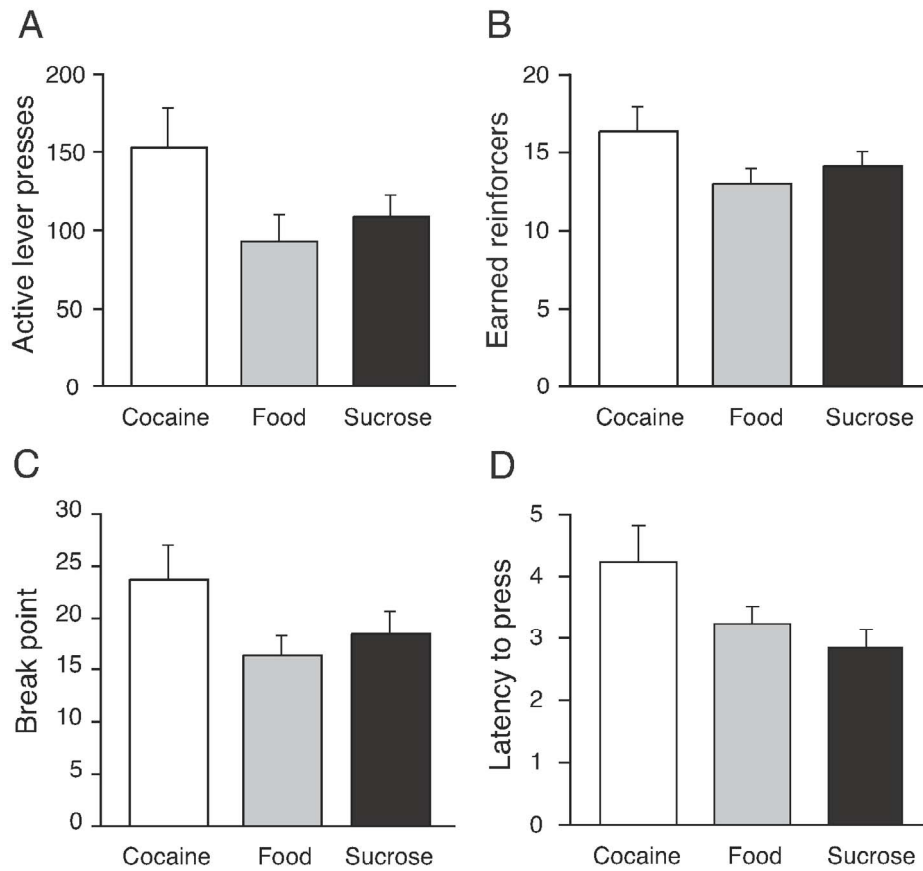
	Days abstinent	Active lever presses	Inactive lever presses	Earned reinforcers
Cocaine	1 day (8)	42.5 ± 2.8	3.0 ± 1.8	38.4 ± 4
	7 days (9)	31.8 ± 9.9	2.8 ± 1.8	21.1 ± 6.4*
	21 days (10)	46.3 ± 2.7	3.3 ± 1.4	32.8 ± 2.9
	90 days (8)	24.5 ± 5.0	0.3 ± 0.3	17.0 ± 3.3**
Food	1 day (11)	57.2 ± 2.4	2.6 ± 1.0	44.0 ± 2.46
	7 days (10)	38.7 ± 4.9	0.0 ± 0.0	32.0 ± 4.3
	21 days (13)	50.0 ± 3.6	2.3 ± 0.8	40.2 ± 4.0
Sucrose	1 day (8)	52.4 ± 4.4	09. ± 0.6	44.0 ± 4.0
	7 days (8)	53.6 ± 4.1	2.9 ± 2.1	42.8 ± 3.3
	21 days (8)	46.6 ± 6.3	3.0 ± 1.9	39.6 ± 4.4

Data are expressed as averaged values for the last day of training (mean ± SEM). Numbers in parenthesis represent number of rats. *P < 0.05 and **P < 0.01 versus 1 day.

Figure legend for supplemental figures.

Supplemental figure 1. Rats self-administering cocaine, food, and sucrose are equally motivated to obtain reinforcers after 21d of abstinence. (A) Active lever presses, (B) reinforcers earned, (C) break point, and (D) latency to re-initiate lever press were not significantly different between Cocaine, Food, and Sucrose rats after 21d of abstinence.

Chen et al. Supplemental Figure 1



Supplemental figure 2. Pairing of yoked cocaine delivery with cues potentiate AMPAR/NMDAR ratio in VTA DA neurons. AMPAR/NMDAR ratio in Yoked + Cue group was 1.22 ± 0.22 , $n = 4$ ($p < 0.05$ versus Naïve). Dotted line represents averaged AMPAR/NMDAR ratio from one-day abstinent Cocaine rats.

