Amphetamine-Enhanced Sensation Seeking and Its Neural Correlates in the Ventral Pallidum

by

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Abstract

Sensation seeking has been studied as a potential characteristic for addiction-prone individuals. In these experiments, we trained rats to make nose-pokes into an “active hole” that produced a flashing light and tone or an “inactive hole” that produced nothing. During testing sessions, we compared the responses of amphetamine-treated rats to responses of vehicle-treated rats. We hypothesized that the stimulus would be inherently rewarding and that amphetamine would increase this effect. Our results showed that, rats did not make more active than inactive nose-pokes after vehicle injection, while amphetamine-treated rats made many more active hole nose-pokes. We also found that the increase in sensation-seeking induced by amphetamine only occurred when rats were given amphetamine on the first day of testing, when the stimuli were more novel. In addition, we measured neural activity within the ventral pallidum, a brain area involved in the processing of rewards and reward-related stimuli. During testing sessions, we recorded ventral pallidal neural activity and assessed whether amphetamine-induced increases in dopamine alter neuronal firing within the ventral pallidum in response to novel stimuli. We found that more ventral pallidal neurons were responsive to the active than inactive nose-poke hole and that amphetamine caused an increase in responsive units. These results indicate that novel stimuli may be processed similarly to food and drugs in the ventral pallidum.

Amphetamine-Enhanced Sensation Seeking and Its Neural Correlates in the Ventral Pallidum

Sensation seeking, or sensory reinforcement, plays an important role in normal and maladaptive behaviors such as compulsive gambling, hyper sexuality, and drug addiction. Individuals that conduct this behavior do so for the mere novel stimuli that follow. Bardo, Donohew, and Harrington (1996) describe how research conducted on United States college students using the Zuckerman's Sensation Seeking Scale found that while only 23% of the "low sensation seekers" had used one or more drugs, 74% of the students labeled as "high sensation seekers" had used one or more drugs. There is also a high correlation between sensation seeking and more liberal attitudes towards promiscuity (Bardo et al., 1996). Researchers are interested in operant sensation seeking because it may be related to behavioral addictions (such as pathological gambling or compulsive eating) and substance abuse in humans (Olsen & Winder, 2009). Therefore, we aim to test the reinforcing properties of novel stimuli by assessing whether or not a rat will nose poke for a tone and flashing light.

In laboratory studies of operant sensation seeking in rats, rats must work for a reward, and instead of being rewarded with food or drugs, they are presented with a novel stimulus such as a flashing light or tone (Olsen & Winder, 2009). The basic premise of creating novelty in an experiment is that the subject does not encounter a similar situation prior to being tested (Hughes, 2007). Laboratory animals find some visual stimuli rewarding even without it being previously associated with a primary reward. Animals with no prior training will respond for the novel stimulus of light illumination (Stewart, 1960). Novelty assessment has been studied experimentally in humans as well. For instance, Bardo et al. (1996) describe how during the visual novelty preference test subjects spent more time looking at a novel than a more familiar stimulus. In an early study of the reinforcing properties of novel stimuli, rats were willing to

press the lever more times for a light stimulus than during sessions when the lever pressing produced nothing. Rats were also willing to work harder (press the lever more times) for a higher light intensity (Stewart, 1960). Therefore, novel stimuli can be rewarding even when the stimuli are not associated with the primary reward such as food or abused drugs.

One noteworthy aspect of the brain's responses to novel stimuli is the similarity between the brain's dopaminergic response to encountering a novel stimulus and its response to experiencing drugs of abuse. The ability of novel stimuli to activate the mesolimbic dopamine system may underlie its rewarding properties, as this effect plays a major role in the rewarding value of drugs of abuse and other natural rewards (Hughes, 2007; Olsen & Winder, 2009). Many arousing events, such as novel stimuli, unexpected rewards, and high-strength auditory and visual rewards cause increased activation of dopamine neurons (Horvitz, 2000).

In a typical "operant sensation seeking" experiment, an animal presses an "active" lever to cause a visual cue such as a flashing light. Animals also have the option to press an "inactive" lever that does not produce a stimulus, and the number of presses on the two levers is compared. In Olsen and Winder's (2009) experiment, responding for a flashing light was similar to responding for cocaine. However, when the experimenters tested either dopamine-knockout mice or mice that had been given nucleus accumbens dopamine-afferent lesions, the mice pressed the active lever fewer times than the dopamine-intact mice for the flashing light (Olsen & Winder, 2009). These results show that the dopamine-intact mice are motivated to receive novel stimuli and that dopamine neurotransmission is required for this response.

Other studies have shown the involvement of the neurotransmitter dopamine in sensory reinforcement through the experimental use of amphetamine. Amphetamine is a dopamine agonist which increases the amount of synaptic dopamine in an animal (Heikkila, Orlansky,

Mytilineou, & Cohen, 1975; Seiden, Sabol, & Ricaurte, 1993). Winterbauer and Balleine (2007) suggest that dopamine promotes the ability of cues to develop reinforcing properties. To study the rewarding value of cues and the involvement of dopamine in their reward value, they performed an experiment in which rats were either given systemic amphetamine injections or vehicle injections prior to testing. The rats had no prior training. During the test, pressing an "active" lever presented a flashing key light stimulus. They found rats would not work for the stimulus during vehicle sessions, but worked avidly for the stimulus after amphetamine administration. Importantly, while the stimulus was not paired with a food or drug reward, the flashing key light that it produced essentially became the primary reward (Winterbauer & Balleine, 2007). Thus, the dopamine played a role in enhancing the reinforcing value of the novel stimulus.

Shin, Cao, Webb, and Ikemoto (2010) also showed that some stimuli, such as flashing lights, become more appealing in the presence of psychomotor drugs such as amphetamine. They found that rats lever pressed more for a flashing light when administered, amphetamine. They also showed that after injecting amphetamine into different brain areas, the most lever pressing occurred when amphetamine was injected into the medial accumbens core, accumbens shell, and especially the olfactory tubercle. In addition, rats pressed the lever more times for a visual signal than for an auditory signal after amphetamine administration. If the amphetamine was co-administered with SCH23390, a D1 dopamine receptor antagonist, the increased arousal to novel visual signals found with amphetamine was significantly decreased (Shin et al., 2010). These studies show that increased dopamine in certain brain areas, specifically the nucleus accumbens and olfactory tubercle, induces rats to work harder for visual stimuli. Together, these findings indicate that unconditioned stimuli become more enticing after administration of psychomotor

stimulants. Consequently, this relationship between novel stimuli and drugs may be important in the study of the initiation of drug taking and how this leads to addiction. Drugs may cause stimuli in the environment to develop a more rewarding effect. They may then attract the drug taker to an environment where he or she can encounter these stimuli and thus reinforce more drug taking.

The activity of dopamine neurons may be involved in an animal's behavioral response to novel stimuli. Dopamine activity in the midbrain is increased with unexpected presentation of stimuli (Horvitz, 2000). Redgrave and Gurney (2006) suggest in their "Re-selection hypothesis" that dopamine neurons in the basal ganglia are involved with action selection by reinforcing the repetition of actions recently performed. By assessing dopamine neuron activity, they conclude that the phasic dopamine response that involves a short burst of firing may be caused by an unpredicted movement or sound in the animal's vicinity. The short-latency phasic firing is caused by unforeseen sensory events that have no primary reinforcing consequences but are significant due to their similarity to reward-related stimuli, their intensity, or their novelty (Redgrave & Gurney, 2006). Therefore, Redgrave and Gurney (2006) propose that the phasic dopamine firing is what leads the animal to re-engage in the behavior that originally caused presentation of the novel stimulus. This leads to the proposal that animals and humans with excessively responsive dopamine systems may purposelessly repeat chunks of behavior associated with distinct sensory outcomes and this could be a mechanism of schizophrenia and the compulsive aspects of disorders such as addiction (Redgrave & Gurney, 2006). Therefore, an imbalance in the neural components involved in responding to novel stimuli may lead to abnormal behavior, but normal neuronal firing may lead to behavior that reflects a novel stimulus's rewarding value.

Little is known about how neurons that receive projections from dopamine-containing structures encode novel stimuli. Hughes (2007) describes how preference for novelty that comes from motivated exploration assumes that the brain pathways involved with other rewards are also involved with the response to novel stimuli. Dopaminergic neurons in the ventral tegmental area of the midbrain targeting structures in the limbic forebrain -particularly the nucleus accumbens – make up the mesolimbic dopamine pathway (Nestler, 2005). The nucleus accumbens (also known as the ventral striatum) makes up the majority of the group of interconnected nuclei in the fore- and mid-brain called the basal ganglia and the striatum contains the most dopamine receptors of any vertebrate brain structures (Humphries & Prescott, 2009). Thus, the mesolimbic dopamine neurons that project to the nucleus accumbens play a significant role in the reinforcing and incentive motivational properties of rewards (Horvitz, 2000).

As a putative result of the dopaminergic input into the nucleus accumbens, the nucleus accumbens exhibits a burst of action potentials in response to salient sensory events in the environment (Horvitz, 2000). One of the structures that the nucleus accumbens sends projections to is the ventral pallidum (Humphries & Prescott, 2009). Electrophysiological recordings show that these nucleus accumbens-ventral pallidum projections show short-duration ventral pallidal neuronal firing when areas corresponding to the nucleus accumbens shell are stimulated (Humphries & Prescott, 2009). Because of these anatomical connections, the ventral pallidum likely plays a significant role in the brain's natural reward pathway which may also be involved with the processing of novel stimuli.

In addition to being an efferent structure of the nucleus accumbens, the ventral pallidum is a target of dopamine neurons from the ventral tegmental area as well as other limbic system structures, and it plays a role in processing natural rewards and in drug addiction (Tindell,

Berridge, Zhang, Pecina, & Aldridge, 2005). Ventral pallidal neuronal firing patterns code reward and corresponding stimuli and may distinguish between the “wanting” and “liking” properties of reward (Smith, Tindell, Aldridge, & Berridge, 2009). Tindell, Berridge, and Aldridge (2004) demonstrated the ventral pallidum’s role in reward processing by showing that the ventral pallidal neuronal firing rate and the number of pallidal firing neurons increased in response to a conditioned stimulus (tone) after rats had learned to associate it with reward (sugar pellet). Goal-directed behavior increased in concordance with the increased ventral pallidal neuronal firing, and rats approached the reward location more when the reward-predicting cue (tone) sounded rather than when the non-predictive cue (control tone) sounded. In addition, ventral pallidal neurons increased in firing in response to a proximal stimulus (feeder click) after treatment with amphetamine, which has been shown to increase the incentive salience of a conditioned stimulus (Robinson & Berridge, 2008). Thus, amphetamine administration enhances the processing of reward cues by increasing the ventral pallidal neuronal firing rate to a cue (Tindell et al., 2005).

Based on the results of these studies, we hypothesize that a novel stimulus is inherently rewarding, and that the rewarding aspect of a novel stimulus will be encoded by the ventral pallidum. We tested this by simultaneously measuring behavioral and neuronal activity of laboratory rats. Since the ventral pallidum is a brain structure involved with reward processing, we predict that more neurons in the rat’s ventral pallidum will fire when the rat pokes for a novel stimulus in comparison to when the rat pokes its nose into the inactive hole. Our second hypothesis is that dopamine up-regulation (through systemic administration of amphetamine) will enhance the rewarding properties of the novel stimulus and cause an increase in the number of responsive ventral pallidal neurons.

Method

Subjects

Twenty-two male Sprague Dawley Rats (Harlan) were used. Upon arrival, they were handled for approximately 10 minutes each day for two days. They were put on reverse light-dark schedules (lights off at 8am and lights on at 8pm) so that they would be most active during the day, and were provided with standard rodent chow and water *ad libitum*. The University Committee on the Use and Care of Animals approved experimental methods.

Apparatus

We conducted the sensory reinforcement testing in a 25X30cm test chamber. The two opposite sides of the chamber which were the longest, were made of plastic and one side had a swinging plastic door in order for the experimenter to place the rat into the chamber. During chamber habituation days, the end walls of the testing chamber consisted of removable metal plates. During testing sessions, two nose-poke holes were placed on one side of the chamber, and a speaker was inserted above these holes near the top of the chamber. On the opposite side of the chamber, there was a red house light that remained in the testing chamber during both the habituation days and during the testing sessions. Two cameras were placed outside of the chamber and were positioned so that they focused on the nose-poke holes. In this way, the rats' behavior could be video recorded. A receiver that would pick up the neuronal signals from the rats' wireless recording electrode was suspended from a shelf on top of the chamber so that it hung down to the top of the chamber. The testing chamber was located within a sound and light attenuating cabinet.

Electrode Design and Implantation

We surgically implanted recording electrodes bilaterally into the ventral pallidum of ten of the twenty-two behaviorally-tested rats six to seven days before testing. Rats were anesthetized using 1.5%-3% isoflurane. We implanted electrodes bilaterally in the posterior ventral pallidum (coordinates: AP-0.6, ML+/-2.5, DV-7.0; Paxinos & Watson, 2007). Each electrode had two bundles (one on each side) of either four or eight tungsten wires (each 25µm). The electrodes had a microdrive that allowed us to move the electrode within the ventral pallidum to record from different sets of neurons during the testing phase. The complex containing the electrode was held in place in the skull with bone screws and dental acrylic. Following the surgery, the rats were given six to seven days to recover.

Behavioral Testing

Behavioral testing began six to seven days after surgery. Ten minutes following a systemic injection of saline, we put the rats into the testing chamber for a 40 minute habituation session. The nose-poke holes and speakers were not presented during this session, which was included so that the rats would become acquainted with the chamber, in the absence of the nose poke holes. After a rat was placed in the chamber the house light would turn on within three seconds and remain on for the entire 40 minute session.

Following this session, rats received either an injection of .75mg/kg of amphetamine (i.p.) or saline vehicle in a counter-balanced fashion. Ten minutes following the injection, we then placed the rats in the testing chamber, which now included two adjacent nose-poke holes and a speaker near the top of the chamber. As with the habituation session, the red house light turned on after three seconds and the rats remained in the chamber for forty minutes. When each rat put its nose in the "active" nose-poke hole, the nose-poke hole light and house light flashed for three seconds (on 0.5seconds, off 0.5seconds) and an 80db tone sounded for three seconds as

well. When the rat poked its nose into the “inactive” nose-poke hole, no stimulus was produced. During the testing sessions, the rat’s behavior was video recorded and the active and inactive nose-poke hole entries were detected using either MED-PC IV (Med Associates Inc., St. Albans, VT) or MTask (Aldridge Lab, University of Michigan). On day two of testing, the rats that received amphetamine on day one were given saline while the rats given saline on day one were given amphetamine. Testing then proceeded as described for the previous testing session. This resulted in four treatment groups: vehicle with no prior treatment, vehicle with prior amphetamine treatment, amphetamine with no prior treatment, and amphetamine with prior vehicle treatment.

Electrophysiology

The electrophysiological testing took place concurrently with the behavioral testing. Ventral pallidal neuronal activity was recorded using DataTask (Aldridge Lab, University of Michigan). We attached a small wireless head-stage to the rat’s electrode implant. This enabled the rat’s neuronal activity to be recorded wirelessly during the testing sessions. Before each recording testing session, we confirmed the presence of neuronal signals from the recording electrode. If no neuronal signal was present on the first attempt, we lowered the electrode 0.5mm or until a signal was detected. Four of the tested rats had eight-channel recording electrodes, while the other eight rats were implanted with 15-channel electrodes.

Neuronal recordings were sorted using Off Line Sorter (Plexon, Dallas, TX). The timestamps of each neuronal waveform, as well as timestamps of active and inactive nose-pokes were imported into Neuroexplorer in order to create perievent raster plots and histograms. More than one reviewer then rated the histograms and perievent raster plots for each unit sorted, and compared them to the corresponding “background” histogram and perievent raster. At least five

trials in the raster plots needed to show an increase (excitation) or severe decrease (inhibition) near stimulus presentation for the unit to be considered responsive. Units that displayed three or less trials during the session were omitted from the analysis. The background was an interval of ten seconds during the session when the rat was not making any nose-pokes. We then determined which units displayed responses to active or inactive nose-pokes. The reviewing was done without knowledge of which unit belonged to which treatment group. For all treatment groups, we recorded the percentage of responsive units (including excitatory responses and inhibitory responses) for active and inactive nose-pokes for each treatment group by dividing the total responsive units by total units sorted for active and inactive hole.

Finally, we made composite histograms for each of the following: for all responsive units (in all treatment groups), and active nose-pokes for each of the following treatment groups: vehicle with no prior treatment, vehicle with prior amphetamine treatment, amphetamine. However, we did not find any responsive units in the amphetamine with prior vehicle treatment group, so no histogram is displayed for this group. We calculated the normalized change of firing rate by using the "background" rate 10s before stimulus onset.

Statistics

For our behavioral analysis, we used a Repeated Measures ANOVA to determine if there was a main effect of drug (amphetamine or saline vehicle), type of nose-poke hole (active or inactive), or if there was an interaction between the drug and type of nose-poke hole. Prior treatment (none, vehicle, or amphetamine) was included in some analysis. P-values of less than 0.05 were considered significant. Tukey's post-hoc comparisons were used to examine significant interactions.

Post-mortem Assessment of Electrode Placement

When testing was complete, the rats were given an overdose of Fatal Plus (0.5ml). Their brains were removed and quick-frozen in a cold isopentane solution. Using a cryostat, the brains were sliced into 50um thick coronal sections and mounted onto charged slides. Histological analysis for the current experiment is ongoing and will involve staining the slices with cresyl violet and assessing the electrode tract to determine if the electrode recorded from the ventral pallidum. Electrode placement targets the ventral pallidum and is based on coordinates established during previous experiments that successfully hit the ventral pallidum.

Results

Nose-Pokes on Day One

We first recorded the number of times during each day one 40 minute testing session that each rat poked its nose into the active versus inactive nose-poke hole. We then compared the number of nose-poke entries into each hole during the testing sessions for the 11 rats that had been given systemic injection of vehicle to the number of nose-poke hole entries during the testing sessions for the 11 rats that had been given a systemic injection of amphetamine. We found that on day one, systemic injections of amphetamine increased nose-poking in the active hole, compared to vehicle (Figure 1, nose-poke hole by drug interaction, $F(1,20)=5.93$, $p<0.05$).

Post-hoc analyses indicated that rats that were treated with vehicle did not display a difference in the number of active versus inactive hole entries, $M_{active}=17.73$, $SD_{active}=15.58$, $M_{inactive}=13.81$, $SD_{inactive}=7.82$, $p>0.05$. However, rats that were treated with amphetamine on day one made more active than inactive nose-poke hole entries, $M_{active}=54.91$, $SD_{active}=58.95$, $M_{inactive}=10.45$, $SD_{inactive}=7.85$, $p<0.05$. The rats that were given amphetamine made more active nose-pokes in comparison to rats treated with vehicle, $M_{amph.}=54.91$, $SD_{amph}=58.95$, $M_{veh.}=17.73$, $SD_{veh.}=15.58$, $p<0.05$. However, there was no significant difference in inactive nose-pokes

between the amphetamine and vehicle-treated rats, $M_{amph.}$=10.45, $SD_{amph.}$=7.85, $M_{veh.}$=13.81, $SD_{veh.}$=7.82, $p>0.05$).

Nose-Pokes after Vehicle Treatment

The rats that received vehicle on day one, received amphetamine on day two, and the rats that received amphetamine on day one received vehicle on day two. Therefore, using only the vehicle testing sessions, we compared the number nose-pokes after no prior drug treatment (rats given vehicle on day one) to the number of nose-pokes after prior treatment of amphetamine (rats given vehicle on day two). We found that there was a trend for interaction of nose-poke hole and prior treatment (none, amphetamine) between the vehicle-treated rats with no prior treatment compared to rats with prior treatment with amphetamine (Figure 2, $F(1,20)=3.47$, $p=0.078$). Thus, the results show that if the rats received amphetamine on the first testing day, it did not significantly impact the number of active or inactive nose-pokes that the rats made on the second day of testing when they received vehicle.

Nose-Pokes after Amphetamine Treatment

We also performed an assessment comparing trials where rats received amphetamine. We compared the number of responses made after amphetamine in rats with no prior treatment to the responses made after amphetamine in rats that had prior vehicle treatment. There was a significant interaction between nose-poke hole and prior treatment (Figure 3, $F(1,20)=4.62$, $p<0.05$).

Post-hoc analyses showed that amphetamine-treated rats that had no prior drug treatment (given amphetamine on day one) made significantly more active nose-pokes than inactive nose-pokes, M_{active}=54.91, SD_{active} =58.95, $M_{inactive}$=10.45, $SD_{inactive}$=7.85, $p<0.05$. However, amphetamine-treated rats that received prior vehicle treatment did not make more active nose-

pokes than inactive nose-pokes, M_{active}=16.00, SD_{active}=19.15, $M_{inactive}$=7.63, $SD_{inactive}$=6.80, *p*>0.05. Rats with no prior vehicle treatment also made more active nose-pokes than rats given prior vehicle treatment, $M_{no\ prior\ tx.}$=54.91, SDno prior tx.=58.95, Mprior tx=16.00, $SD_{prior\ tx.}$=19.15, *p*<0.05.

Therefore, while systemic injection of amphetamine on the first day of testing clearly increased the number of active nose-pokes that the rats made while having no effect on inactive nose-pokes, this effect only occurred if the rats were given amphetamine on day one. If the rats were tested with amphetamine, but with prior treatment of vehicle (given vehicle on day one and amphetamine on day two) the amphetamine did not have an effect on the number of active versus inactive nose-poke entries that the rats made.

Neuronal Response

For our assessment of ventral pallidal units that responded to the active or inactive nose-poke holes, we found that there were more responsive units for active compared to inactive hole nose-pokes (Table 1). Responsive units included those with excitatory responses (increase in firing frequency, Figure 4) and inhibitory responses to the nose-poke hole (decrease in firing frequency, Figure 5). The percentage of responsive units relative to total units assessed was relatively similar for both the vehicle group with no prior treatment (Active hole: 26.92%, Inactive hole: 5.56%) and the vehicle group with prior amphetamine treatment (Active hole: 27.78%, Inactive hole: 9.09%). Sessions for rats that received amphetamine but no prior treatment showed the highest percentage of responsive units relative to total units for the active hole (Active hole: 57.14%, Inactive hole: 0%). Rats treated with amphetamine but prior vehicle treatment showed no responsive units for either the active hole or inactive hole.

We produced a composite histogram to demonstrate the frequency of all responsive units when the rats made active nose-pokes (Figure 6) and another for when the rats made inactive nose-pokes (Figure 7). The firing rate remains fairly constant, even during stimulus presentation, for the histogram showing inactive nose-pokes, but the firing rate peaks during stimulus presentation for the histogram of active nose-pokes. Because there were so few inhibitions, we made composite histograms to show all excitations that occurred for all treatment groups except the amphetamine with prior vehicle treatment group, because this group showed no responsive units for any sessions. For the amphetamine with no prior treatment group (Figures 8), the composite histogram demonstrating the excitations in response to active hole show peaks that occur before the nose-poke whereas the histograms for both vehicle treatment groups show peaks that occur after the nose-poke (Figures 9 & 10).

Discussion

The focus of our behavioral assessment was to determine if the rats would work for a novel, unconditioned stimulus, based on our hypothesis that a novel stimulus itself is inherently rewarding. We predicted that on the first day of testing, the rats would make more active hole nose pokes, which would cause a flashing house and nose-poke hole light and a tone to sound, than inactive nose pokes which would produce no stimulus. Since amphetamine increases synaptic dopamine transmission (Heikkila et al., 1975; Seiden et al., 1993), and an increase in dopamine increases the reinforcing value of novel stimuli (Shin et al., 2010), we expected that the group of rats that received amphetamine before testing would make more active hole nose-pokes than the group of rats that received saline vehicle before testing.

On the first day of testing, the stimuli elicited by the active nose-poke hole were truly novel, so to assess whether or not the rats found the novel stimuli rewarding, we first looked at

only their first day's performance. The results showed that rats treated with vehicle on day one did not make more active hole nose-pokes. However, the rats treated with amphetamine on the first day of testing made significantly more active hole nose-pokes than inactive hole nose-pokes. Thus, without alteration of dopamine levels, our stimulus was not reinforcing, but after amphetamine administration, the novel stimulus became substantially reinforcing. This was presumably through an enhancement of its incentive value, which increased "wanting" for the stimulus (Robinson & Berridge, 2008). Inasmuch as amphetamine increases dopamine (it also increases other neurotransmitters as well), we believe that this effect is due to dopamine-mediated enhancement of incentive value.

Amphetamine-treated rats responded more for the novel stimulus than vehicle-treated rats, but the amphetamine treatment had no effect on inactive hole nose-pokes. This shows that the increased dopamine neurotransmission, caused by injection of amphetamine, made the rats more responsive to novel stimuli, but did not cause them to make more nose-pokes to a hole that produced no novel stimuli. This shows that the amphetamine did not simply cause the rats to make more nose-pokes in general, but that they discriminated between the nose-poke holes and put more effort into nose-poking for novel stimuli. These results suggest that increased dopamine specifically influences the rewarding properties of novel stimuli, and this effect is not simply due to amphetamine-induced increases in activity.

While the stimulus produced on the first day of testing is considered the most "novel" since the rats had no prior exposure to it, we counterbalanced the drug treatments for the groups by giving the rats amphetamine on day two if they had received vehicle on day one and giving the rats vehicle on day two if they received amphetamine on day one. For vehicle-treated rats, the trend for significant interaction between nose-poke hole and prior treatment type suggests that in

the future, by adding more subjects to the study, the fact that we gave rats amphetamine when they experienced the stimuli as being novel may affect their nose-poking the next day when only given vehicle. This would show that the drug increased the reinforcing effect of cues enough so that during the next exposure to the stimuli, rats would make more active hole nose-pokes even without the stimuli being as novel and with no drug exposure.

We made the same assessment with testing sessions where the rats were treated with amphetamine. We found that rats given amphetamine with no prior treatment made more active hole nose-pokes than the rats that received prior treatment of vehicle. Thus, the amphetamine only affected the incentive value of the stimulus when the stimulus was relatively novel.

This effect can be generalized to humans and may demonstrate the role of novel stimuli in addiction. A drug-taker encounters certain cues in the drug-taking environment, which among other things may involve light-up bar signs, the sights and sounds inside a bar, a pill bottle, or a cigarette lighter. All of these are examples of cues that normally by themselves are not thought to have a rewarding value, however our experimental results suggest that if they are fairly novel to the drug-taker and in the environment during drug administration, they will become more reinforcing. At a later time, even if the person is not on the drug, the cues will attract him or her, and this will put the person back in the environment and potentially lead to repeated drug use.

Our experiment used amphetamine and since amphetamine is known to increase dopamine neurotransmission (Heikkila et al., 1975; Seiden et al., 1993), this suggests that an increased amount of dopamine is involved with the reinforcing effect of novel stimuli. In the future we may do the experiment again, but instead of amphetamine, use a dopamine antagonist to see if there is a decrease in inactive nose-pokes relative to vehicle with a decreased amount of dopamine. Alternatively, we could treat rats with a dopamine antagonist with amphetamine to

determine if the results we found with amphetamine are cancelled out. This would show that our results are specific to the increase in dopamine.

In our experiment, we had no way of distinguishing the rat's preference for the flashing light from the tone, so to determine whether a visual cue or an auditory cue is more reinforcing, in the future, we could perform the same experiment with twice as many rats so that half of each treatment group nose-poked in the active hole for a flashing light and the other half of the rats nose-poked for a tone. This would allow us to determine whether amphetamine causes a novel visual stimulus or a novel auditory stimulus to be more reinforcing. Since our experiment uses systemic injection of amphetamine, a future experiment along with the results by Shin et al. (2010) that distinguishes between auditory and visual stimuli, may show that visual and auditory novel stimuli are processed in different brain areas.

Although further analysis is needed to confirm the electrode placement in the ventral pallidum and a larger subject pool is necessary to allow for the assessment of more units, our current results suggest that our hypothesis that neurons in the rat's ventral pallidum fire more frequently when the rat pokes its nose for novel stimuli than when it nose-pokes for the inactive hole, was supported. Units for the amphetamine but no prior treatment session also had a higher percentage of responsive units relative to total units, so this supports our other prediction that dopamine up-regulation would increase the number of responsive ventral pallidal neurons to novel stimuli. Interestingly, the composite histograms that we produced for both vehicle treatments show peaks that occur after the nose-poke, however the composite histogram for the amphetamine-treated rats with no prior treatment shows a large peak just before the nose-poke. Amphetamine is involved with change in ventral pallidal firing rate in response to reward cues (Tindell et al. 2005). Therefore, our findings may reflect the motivational properties of novel

stimuli and show that they are processed similarly in the ventral pallidum to primary rewards such as food and drugs.

The amphetamine-induced change in firing rate exhibited by ventral pallidum neurons, when the rat nose-pokes for a novel stimuli, may be part of the mechanism that leads it to find the novel stimuli rewarding enough to nose-poke more in the active than inactive hole. For vehicle trials, the fact that we found there to be higher percentages of responsive neurons for the active hole compared to inactive hole does not correspond to our behavioral results where on vehicle trials, the rats showed no difference in nose-pokes for active versus inactive holes. This may suggest that other parts of the brain may possibly be involved with processing the value of novel stimuli, and while ventral pallidal neurons tended to be more responsive to novel stimuli, neuronal firing of a different brain structure may be determining the rat's behavior.

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Table 1

Percentage of Responsive Units to Total Units Sorted

Treatment	Active Hole	Inactive Hole
Vehicle: No Prior Treatment	27%	5.56%
Vehicle: Prior Amphetamine Treatment	27.78%	9.09%
Amphetamine: No Prior Treatment	57.14%	0%
Amphetamine: Prior Vehicle Treatment	0%	0%

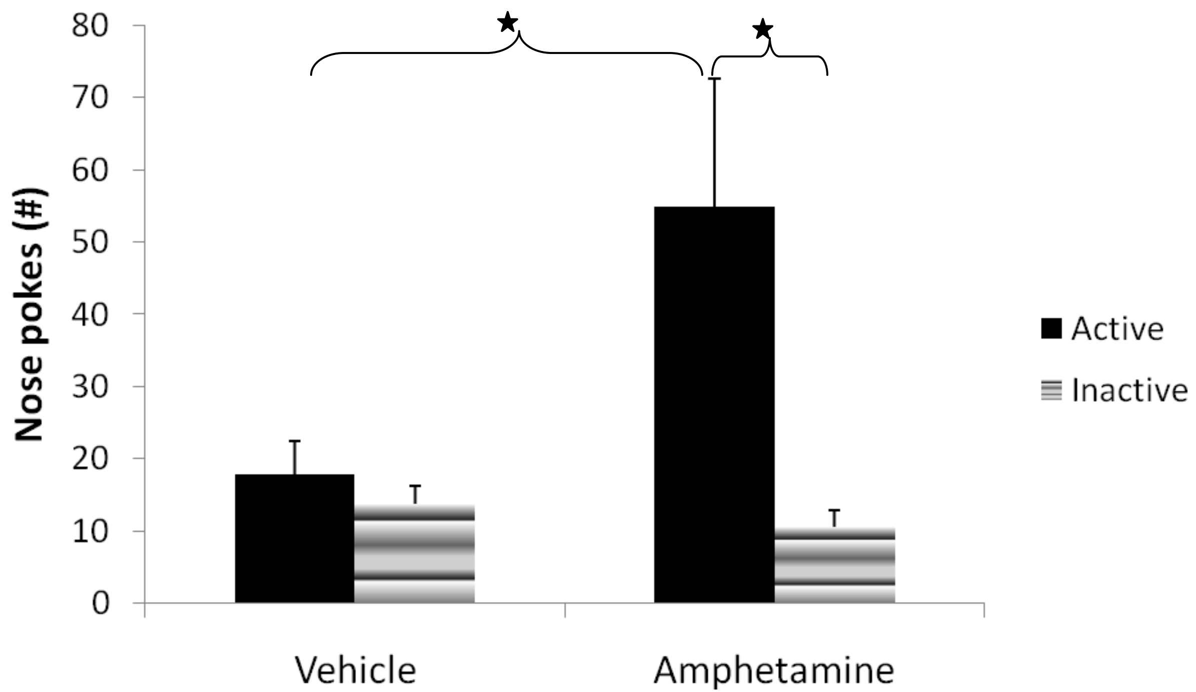

Figure 1. Day One Active vs. Inactive Nose-pokes for Vehicle and Amphetamine Groups. Rats treated with amphetamine made more nose pokes in the active than inactive hole, $p<0.05$ than vehicle-treated rats. There was a significant interaction between nose-poke hole and drug treatment, $F(1,20)=5.93$, $p<0.05$. Error bars represent the standard error. ★=p<0.05

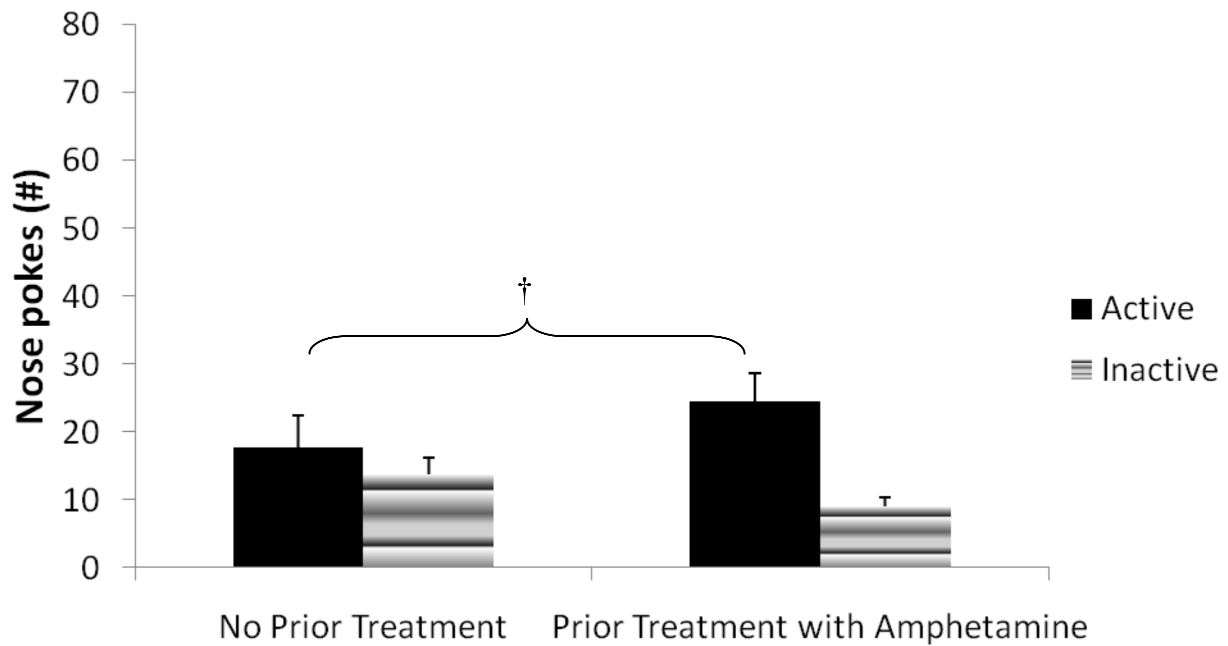

Figure 2. Comparison of Active and Inactive Nose-Pokes for Different Vehicle Treatments. There was a trend for a significant interaction between prior treatment type and nose-poke hole when the rats were tested with vehicle treatment, $F(1,20)=3.47$, $p=0.08$. Error bars represent the standard error. †=trend for significance

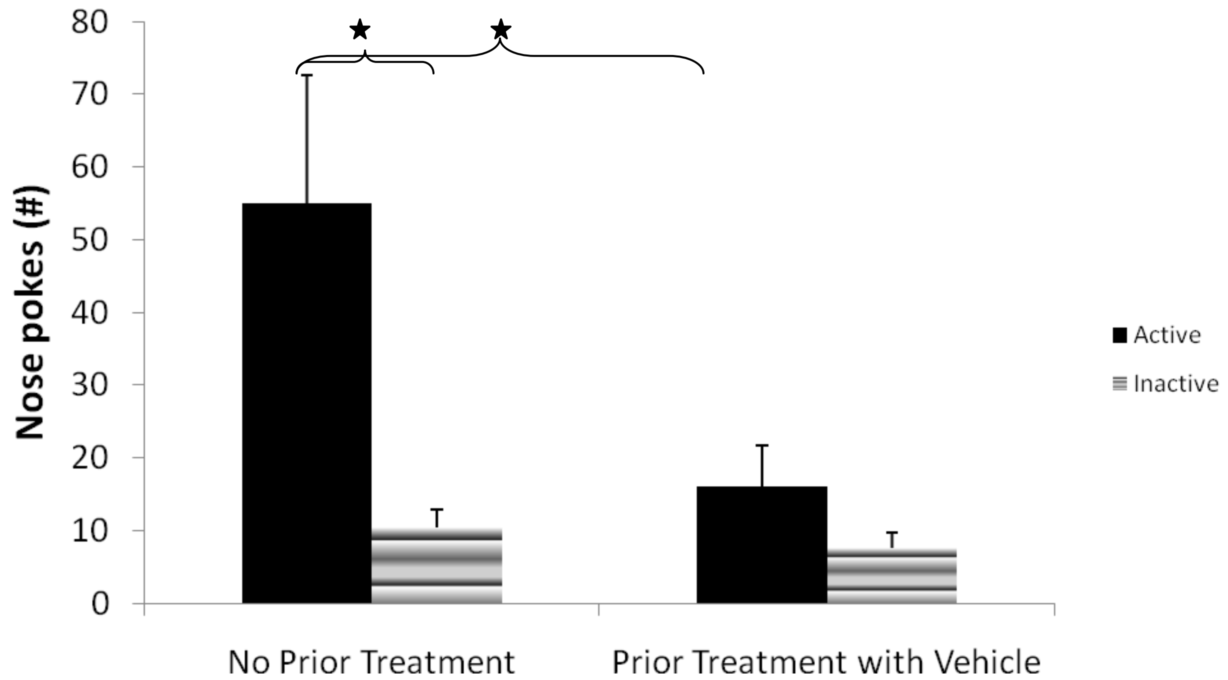

Figure 3. Comparison of Active and Inactive Nose-Pokes for Different Amphetamine Treatments. Rats treated with amphetamine but no prior treatment nose-poked for the active hole more than the inactive hole, $p<0.05$. For amphetamine-treated rats, there was a significant interaction between prior treatment type and nose-poke hole, $F(1,20)=4.62$, $p=0.04$. Error bars represent the standard error. ★=p<0.05

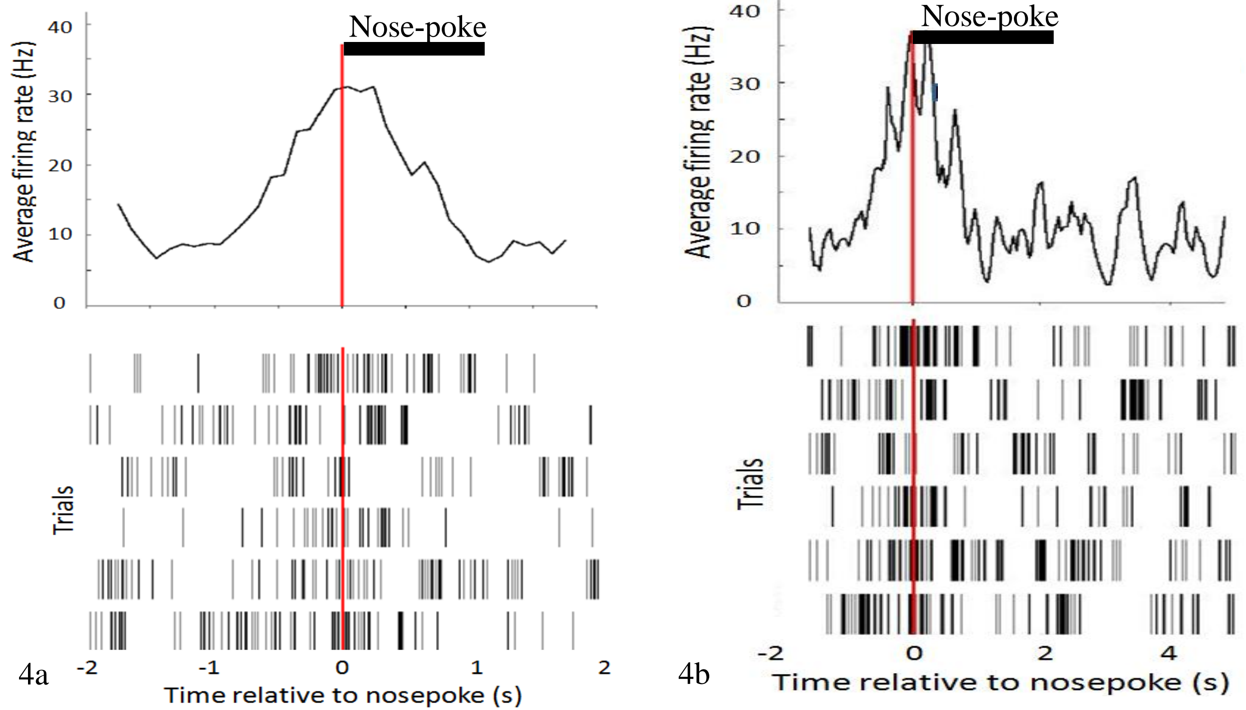

Figure 4. Sample Histograms (top) and Rasters (bottom) for a Ventral Pallidal Unit that Exhibited an Excitatory Response to an Active Hole Nose-Poke for a Vehicle-Treated Rat with No Prior Treatment. The line designates the time of nose poke at t=0seconds. a)The bin size was 50milliseconds, and a 4s time window is shown. b) The bin size was 100ms, and a 7s time window is shown. The vertical marks on the raster represent neuronal action potentials that are composited in the histogram.

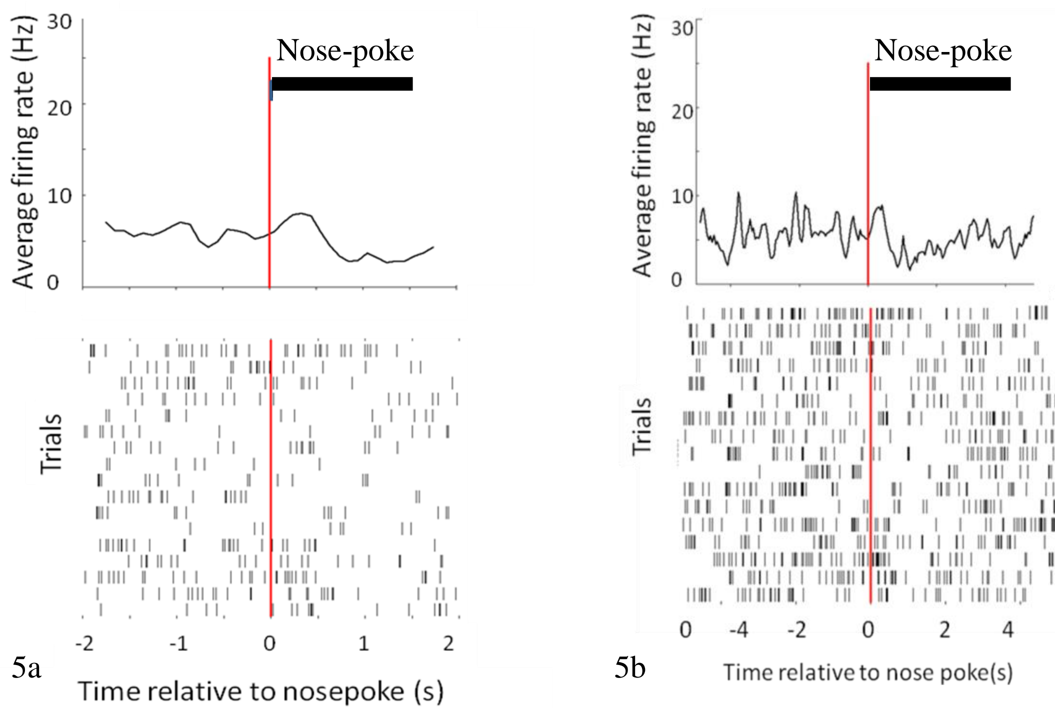

Figure 5. Sample Histogram (top) and Raster (bottom) for a Ventral Pallidal Unit that Exhibited an Inhibitory Response to an Active Hole Nose-Poke for a Vehicle-Treated Rat Prior Treatment of Amphetamine. The line designates the time of nose poke at t=0seconds. a)The bin size was 50milliseconds, and a 4s time window is shown. b) The bin size was 100ms, and a 10s time window is shown. The vertical marks on the raster represent neuronal action potentials that are composited in the histogram.

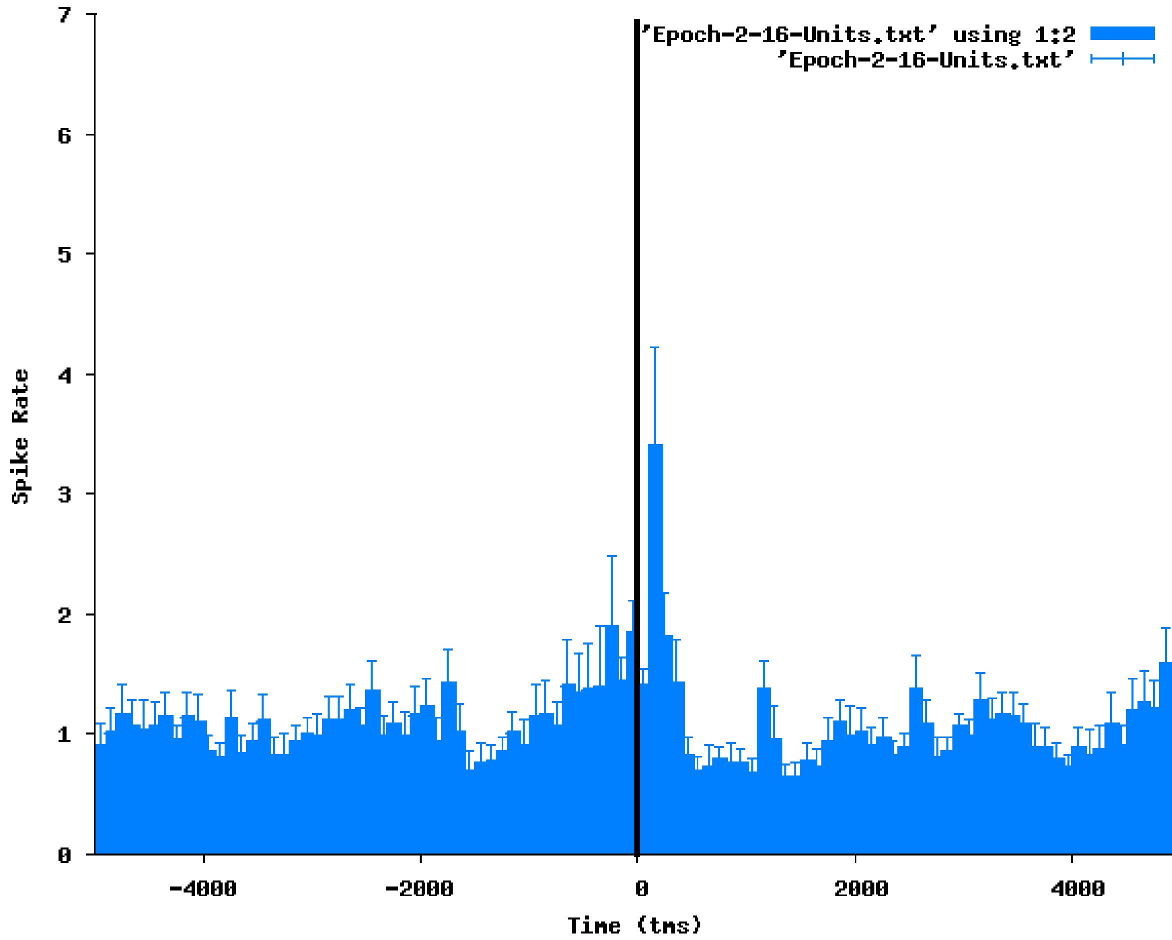

Figure 6. Composite Histogram of the Neural Response to an Active Hole Nose-Poke. The line designates the time of nose poke at t=0seconds. Units that exhibited a response (excitations or inhibition) to either active or inactive hole are included.

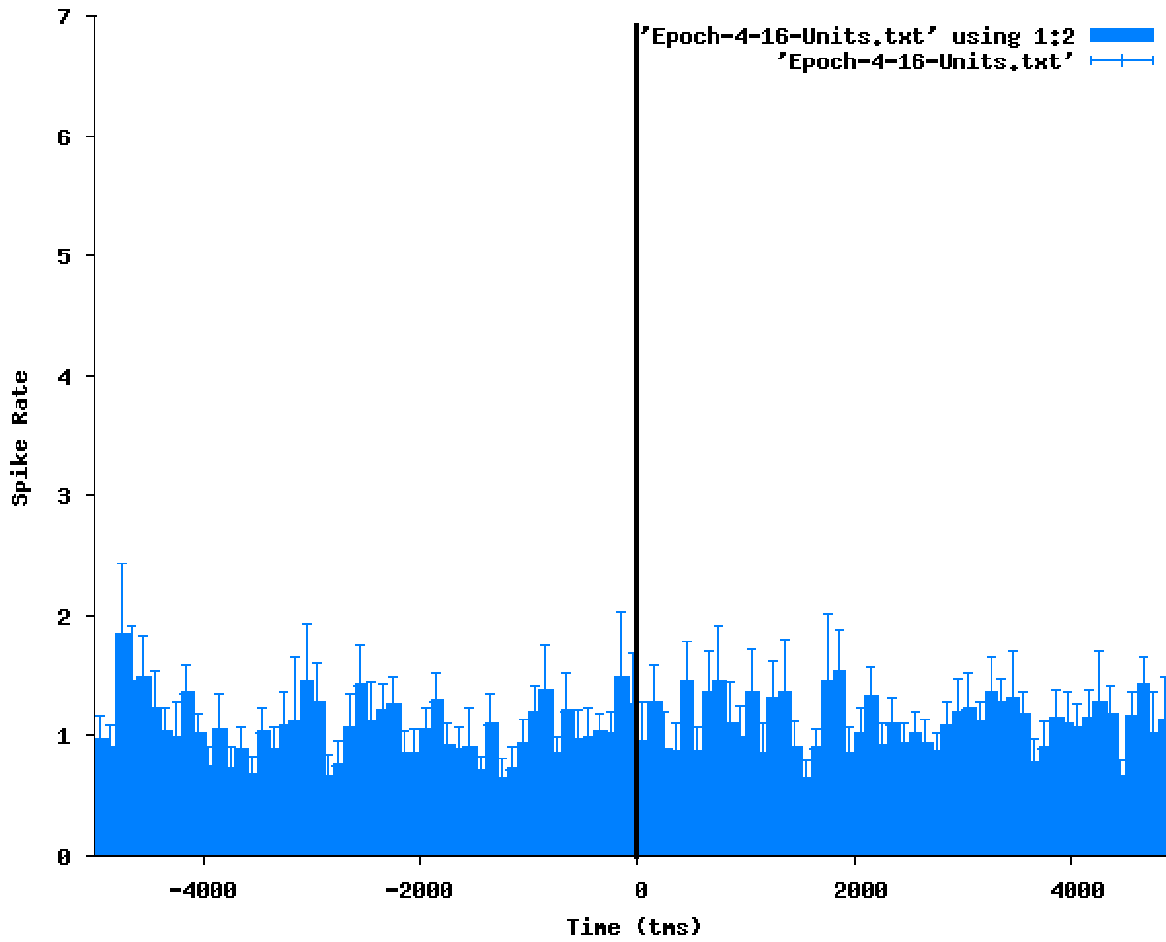

Figure 7. Composite Histogram of the Neural Response to an Inactive Hole Nose-Poke. The line designates the time of nose poke at t=0seconds. Units that exhibited a response (excitations or inhibition) to the inactive hole are included.

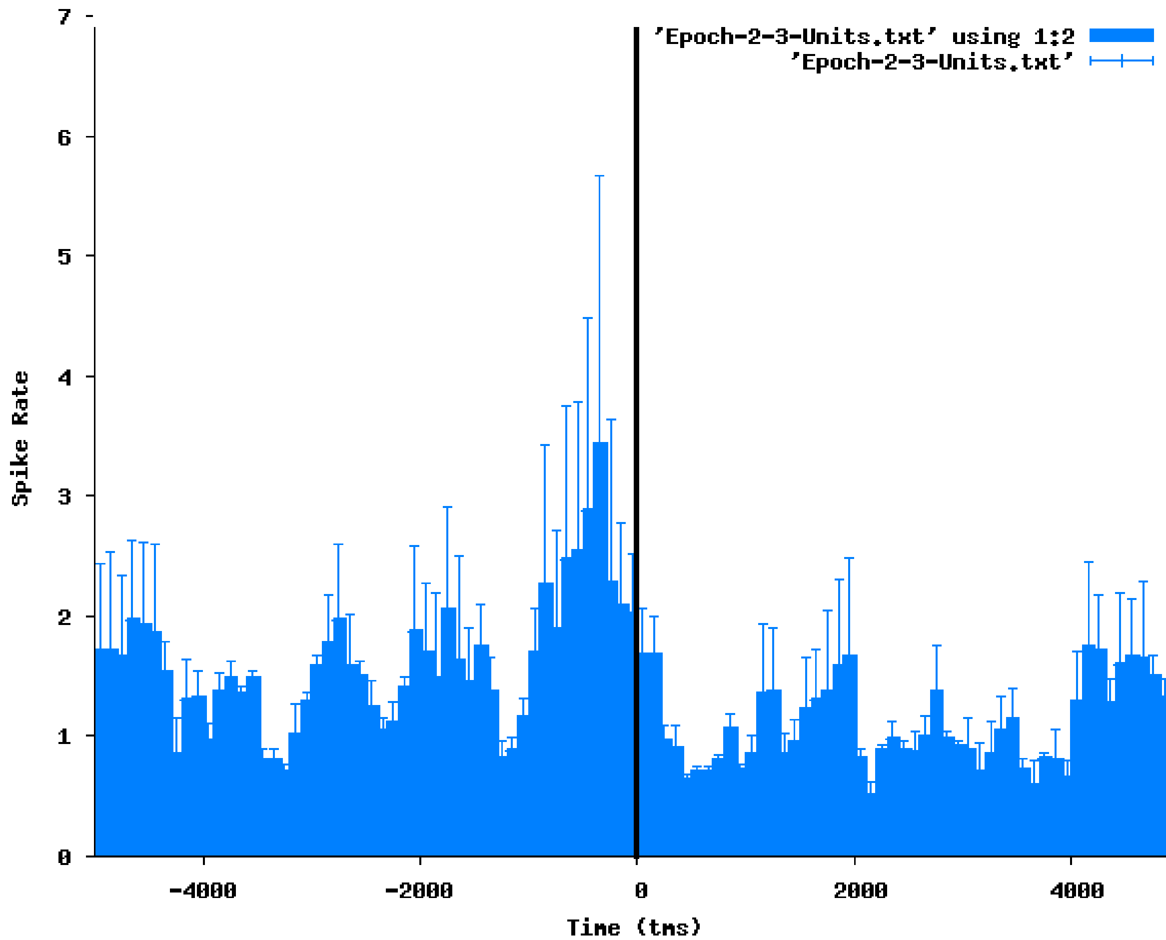

Figure 8. Composite Histogram of all Units with Excitatory Responses to the Active Nose-Poke Hole During Testing Sessions Where Rats were Treated with Amphetamine and had No Prior Treatment. The line designates the time of nose poke at t=0seconds.

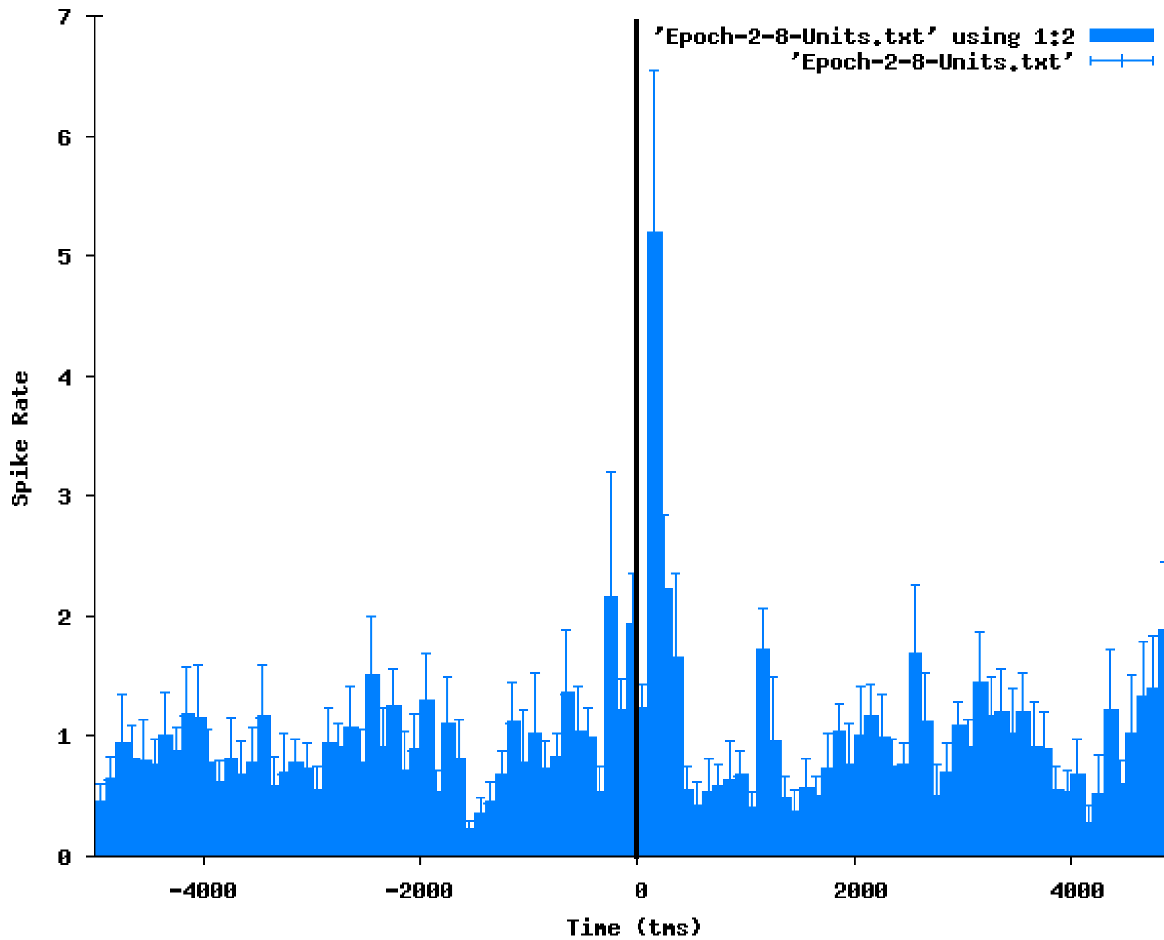

Figure 9. Composite Histogram of All Units with Excitatory Responses to the Active Nose-Poke Hole During Testing Sessions Where Rats were Treated with Vehicle and had No Prior Treatment. The line designates the time of nose poke at t=0seconds.

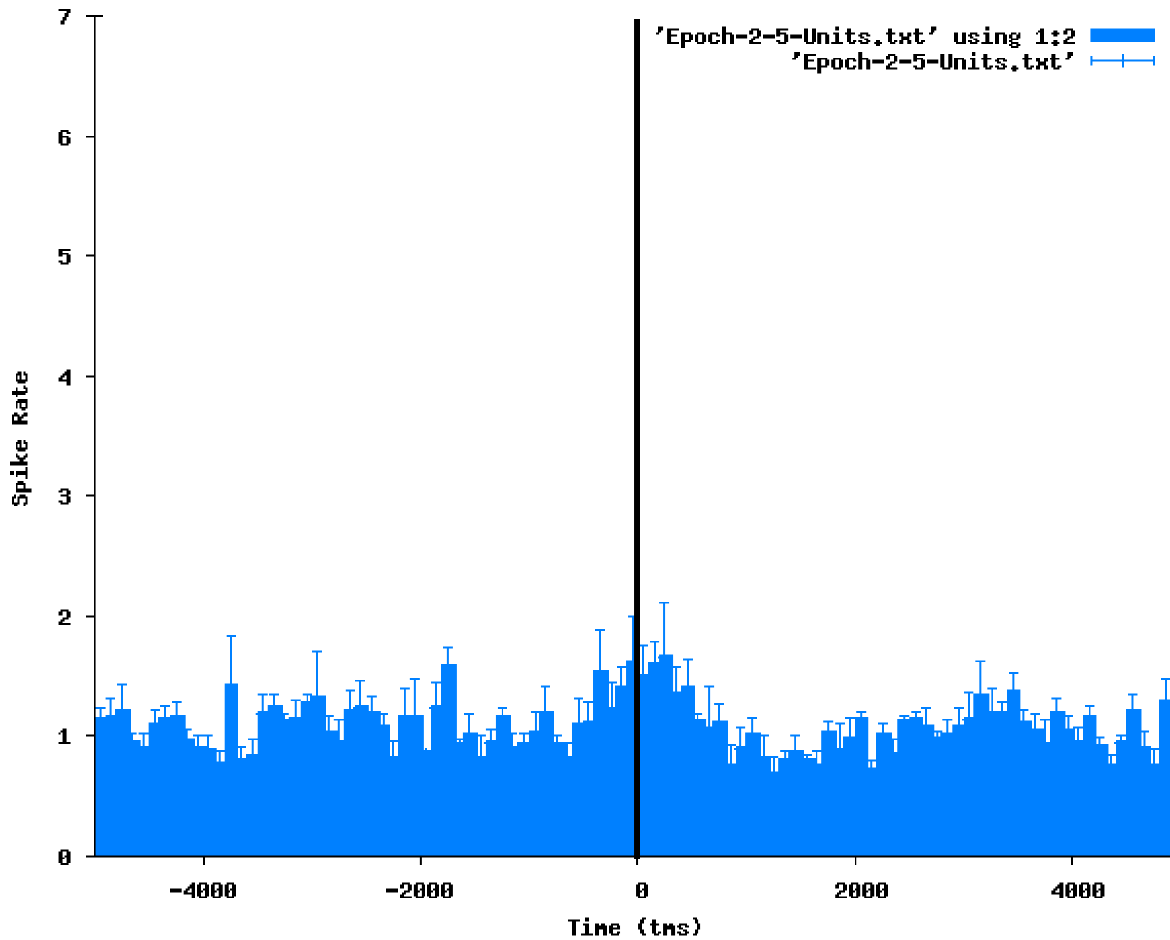

Figure 10. Composite Histogram of All Units with Excitatory Responses to the Active Nose-Poke Hole During Testing Sessions where Rats were Treated with Vehicle and had Prior Amphetamine Treatment. The line designates the time of nose poke at t=0seconds.