The serotonin 5-HT$_{1B}$ agonist sumatriptan increases aggressive behaviors in adult male rats in a neutral cage setting

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Aggressive behavior is defined as that in which an individual aims to harm another person, with the intent to physically or psychologically wound or kill. Aggression – whether unprimed or primed – is thus a trait of interest in both criminal behavior and every day mood management. Previous research (using the established intruder-scenario model) has reported high levels of serotonin and dopamine in the nucleus accumbens of adult male rats, both during and after aggressive bouts. Since the intruder-scenario could have a priming effect on aggression, this study for the first time investigated aggression in a neutral cage, only "priming" with the serotonergic agonist sumatriptan in an attempt to establish a potential connection between levels of nucleus accumbens serotonin and aggressive behavior. Thus, a total of eight male rats were randomly split into four pairs and tested for aggressive behaviors in a single 30-minute session. The pairs each consisted of one saline-infused rat versus one sumatriptan-infused rat. While the results showed non-significant effects of sumatriptan for some aggressive behaviors, sumatriptan-infused rats showed significantly higher occurrences of chasing and clinch attacking as compared to saline controls. Interestingly, both of these behaviors occur towards the beginning of an aggressive bout, suggesting that sumatriptan may influence the initiation of aggressive behavior. More research is necessary to confirm and expand these initial findings.

Abbreviations: 5HT – serotonin; 5-HIAA – hydroxyindolacetic acid; NA – Nucleus Accumbens; PFC – Prefrontal Cortex; A3GH

Keywords: Aggression; Neutral Paradigm; Nucleus Accumbens; 5-HT Agonist; Sumatriptan

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Introduction

Aggressive behaviors play a large role in many types of human interactions. For instance, criminal behaviors, such as theft, assault, rape, and even murder – while all having different motivations and degrees to which they are prompted or escalated – are all crimes involving aggression. Aggressive behavior is defined as that in which an individual aims to harm another person, with the intent to physically or psychologically wound or kill (Aronson, 2007). It is thus important to evaluate and understand where aggressive behaviors originate and the potential triggering mechanisms for aggression.

The role of serotonin (5-HT) in aggressive behavior has been studied in a wide variety of species, including humans (e.g., Berman et al., 1997; Kravitz and Huber, 2003; Summers et al., 2005). Classically, it had been thought that increases in 5-HT may inhibit aggressive behaviors; this inverse relationship between 5-HT and aggression has been coined the "serotonin deficiency hypothesis" of aggression. This hypothesis had been based on research that found reduced levels of 5-hydroxyindolacetic acid (5-HIAA, 5-HT's metabolic product) in the cerebrospinal fluid of excessively aggressive and violent human subjects (see Berman et al., 1997, for review). Some studies in animals have also confirmed the inverse relationship between low 5-HT levels and increased aggression (or high 5-HT levels and reduced aggression; Mongillo et al., 2014; Kohlert et al., 2012; Raleigh et al., 1984). However, research into the role of specific 5-HT receptors (e.g., the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors) presents a more nuanced view.

In most of the literature linking the 5-HT $_{1A/1B}$ receptors to aggressive behavior it has been shown that the pharmacological activation

of the 5-$HT_{1A/1B}$ receptors potently suppresses aggressive behaviors in most animal models studied (for review, see de Boer and Koolhaas, 2005 and Olivier and van Orschoot, 2005). Further, knocking out the 5-HT_{1B} receptor in mice produces exaggerated responses to stress (Groenik et al., 2003), and advanced aggressive behavior (Olivier and Young, 2002; Saudou et al., 1994), indicating that the 5-$HT_{1A/1B}$ receptors normally function as agents to reduce stress and/or aggression. However, activation of the 5-$HT_{1A/1B}$ receptors via systemic administration powerfully reduces the release of 5-HT; it does not potentiate it. Thus, an understanding of the mechanism of action of the 5-$HT_{1A/1B}$ receptors is necessary to resolve the conflicting ideas regarding serotonin's role in aggression.

Pineyro and Blier (1999) have indicated that 5-$HT_{1A/1B}$ receptors have double localization in the brain: they are presynaptic inhibitory receptors (autoreceptors) that regulate the release of 5-HT (5-HT_{1A} autoreceptors are located somatodendritically within the raphé nuclei, while 5-HT_{1B} autoreceptors are located on serotonergic axon terminals in areas of projection, as well as within the raphé; see Olivier and van Oorschoot, 2005), and they can also be inhibitory postsynaptic heteroreceptors on several types of target neurons in corticolimbic regions. Because of this double localization, 5-$HT_{1A/1B}$ receptor agonists, when systemically administered, will exert dual effects on both presynaptic and postsynaptic sites; they should inhibit 5-HT transmission when acting at autoreceptors, and effectively mimic the release of 5-HT at postsynaptic sites in brain regions other than the raphé nuclei (Olivier and van Oorschoot, 2005; de Boer and Koolhaas, 2005). Therefore, the overall effect of systemic administration results from the combined effect of decreased 5-HT release from the raphé and direct stimulation of the postsynaptic receptors, which apparently results in overall decreased 5-HT in the brain.

Given that systemically-delivered 5-$HT_{1A/1B}$ receptor agonists can exhibit these dual effects, much previous research has been conducted attempting to determine whether the presynaptic or postsynaptic effects of 5-HT are more implicated in aggressive behaviors overall (Miczek et al., 1998; De Almeida et al., 2001; de Boer and Koolhaas, 2005). It may very well be that a transient decrease in 5-HT neurotransmission (via activation of 5-$HT_{1A/1B}$ autoreceptors located *both* in the raphé nuclei (5-$HT_{1A/1B}$ receptors) and at forebrain sites (5-HT_{1B} receptors) could be responsible for some of the anti-aggressive effects seen via administration of 5-$HT_{1A/1B}$ receptor agonists. However, investigations assessing aggressive behavior following the activation of 5-$HT_{1A/1B}$ receptors specifically within forebrain regions via direct microinfusion (rather than of agonists being delivered systemically) have been few and varied; in such cases, these agonists were found to either decrease (Cologer-Clifford et al., 1997; Ferris et al., 1999) or increase (De Almeida and Lucion, 1997) aggression, depending upon dosage and area of administration. Intriguingly, Simon et al., (1998) found that the ability of 5-$HT_{1A/1B}$ agonists, alone or in concert, to modulate aggressive behavior in mice was differentiated by region of administration (e.g., lateral septum, medial preoptic area) and hormonal status of the animal. Apart from the study by Simon et al., (1998), most of these effects were shown to be mediated by agonists acting at 5-HT_{1A} receptors alone, or by the combined effects of agonists acting at 5-$HT_{1A/1B}$ receptors in concert with each other.

As results are mixed, this calls into question the role of 5-HT in specific regions of the brain related to aggression: While systemic application of 5-$HT_{1A/1B}$ receptor agonists seems to decrease aggression overall, this may be due to effects at the presynaptic autoreceptors, particularly those found in the raphe nuclei in the case of 5-HT_{1A} receptors, causing a global decrease in the release of 5-HT. However, given the mixed results of direct forebrain infusion discussed above, it may be that 5-$HT_{1A/1B}$ receptors at specific brain sites may promote or inhibit aggression in a site-specific way. Further, to our knowledge, no previous studies have indicated an effect of selective 5-HT_{1B} receptor agonists administered solely to forebrain regions on aggressive behaviors.

The nucleus accumbens (NA) is a brain area commonly related to aggression, not only in rodent models, but in human psychopathology, as well. Spoont (1992) observed correlations

between levels of 5-HT in the NA and predispositions to pathological conditions involving aggression, such as acts of violence and suicide. In a rodent model, van Erp and Miczek (2000) tested aggressive behaviors during a thirty-minute (resident-intruder) fight period (described below), and reported the levels of 5-HT and dopamine obtained via microdialysis in both the NA and the prefrontal cortex (PFC) during the bout. The results indicated a significant increase in 5-HT response in the NA both during and after aggressive behavior (van Erp and Miczek, 2000). Since 5-HT increases in the NA both during and after an aggressive bout, it stands to reason that the administration of a selective serotonergic agonist directly into the NA may mimic the observed increase in serotonin release that occurs during an aggressive encounter.

Fletcher and Korth (1999) have indicated the presence of the 5-HT_{1B} receptor in the NA, particularly within the lateral shell of the NA. Theoretically, if the 5-HT_{1B} receptor is predominant in the NA, then actions of released 5-HT would be mediated at least in part via this receptor, either at its presynaptic (autoreceptor) location or the postsynaptic heteroreceptor. The 5-HT_{1B} receptors located particularly within the lateral shell have been implicated in the stress response of animals (Furay et al., 2011; Nair et al., 2013). Furay and colleagues (2011), in particular, found that daily social defeat stress affected mRNA levels of the 5-HT_{1B} receptor specifically in the nucleus accumbens shell, indicating that the 5-HT_{1B} receptor may be at least somewhat connected to social and/or aggressive/stressful stimuli. The above evidence seems to suggest that an increase in 5-HT in the lateral accumbens shell may at least in part mediate aggressive behavior, and this may be due to the actions of 5-HT on pre- or postsynaptic 5-HT_{1B} receptors. However, direct testing of the effects of local administration of a 5-HT_{1B} agonist or antagonist to the NA shell on aggressive behavior has not yet occurred.

Previous animal studies conducted on aggression have all used the intruder-scenario as a model for testing such behavior (Koolhaas et al., 2013; Olivier and Young, 2002; Tidey and Miczek, 1996). The intruder scenario calls for a situation in which a male rat (the resident) develops so-called cage dominance via mating with an ovariectomized female for one week in his home cage. Then, during the aggression-testing period, the female is removed while an unfamiliar, naïve male rat (the intruder) is placed in the cage with the resident. The resident is then allowed to engage in an aggressive altercation with the intruder (Koolhaas et al., 2013). However, in an intruder-scenario, resident rats can be considered to be "primed" for aggression via the establishment of cage dominance. Thus, if a male rat is able to establish a salient reason for aggression, then this paradigm does not necessarily test "unprimed" aggressive tendencies of individual rats, that is, whether one rat is more likely to engage in aggression without direct cause than another rat. Therefore, a different paradigm is needed to address more fully the neural causality (if any) of unprimed aggression that may occur in individuals with aggressive tendencies, thus requiring a new experimental paradigm: the neutral cage. All rats are subjected to this environment as a new environment, without being allowed to previously explore the chamber. Since rats would have no previous exposure to the environment or one another, familiarity with the cage as a "home" cage does not exist, and, therefore, no "reason" for aggression should also potentially exist.

In sum, the NA could be a potential center for the control of some aspects of aggressive behavior. Additionally, it could be that the link between aggressive behavior and high levels of 5-HT in the NA may be mediated via the 5-HT_{1B} receptor, either pre- or post-synaptically, although, intuitively, it should more likely be mediated postsynaptically, as activating the presynaptic autoreceptor should lower extracellular 5-HT levels. Our goal in this study was not only to examine aggression, but to manipulate a part of the neural circuit involved in this behavior that had not previously been tested, that is, the 5-HT_{1B} receptor in the NA. Further, because the intruder-scenario could have a priming effect on aggression, this study, for the first time, investigates aggression in a neutral cage, only "priming" with a 5-HT_{1B} specific agonist, sumatriptan, rather than giving a behaviorally salient reason for aggression. The combination of the neutral cage and sumatriptan

delivered directly to the NA will, hopefully, provide insight into the potential for a predisposition to unprimed aggression as well as the mechanisms of 5-HT mediation of aggressive behavior.

Thus, in this study, the potential link between the activation of 5-HT_{1B} in the NA and aggression were tested via application of the specific 5-HT_{1B} agonist, sumatriptan, directly to the NA shell. Since increases in 5-HT are associated with aggressive behavior (van Erp and Miczek, 2000), we hypothesized that sumatriptan may act more on postsynaptic 5-HT_{1B} receptors in the NA and could, potentially, cause an increase in aggression. However, if we there was a decrease in aggression after administration of sumatriptan, then perhaps presynaptic 5-HT_{1B} receptor actions may be more prevalent in this area, and it would suggest that increases in 5-HT seen during aggression are not normally attenuated via the 5-HT_{1B} receptor. If activating the 5-HT_{1B} receptor in the NA elicits aggression (suggesting postsynaptic actions) in a neutral scenario, then this receptor could be evaluated as a potential contributor to aggressive tendencies. This would help advance research in criminal behavior, by providing avenues for future research in identifying those who may naturally have a predisposition to aggression. Therefore, we hypothesized that, if sumatriptan acts on postsynaptic 5-HT_{1B} receptors in the NA, then it will cause aggressive behavior in male rats.

Material and Methods

Subjects

Eight, male Long-Evans rats (Harlan; Indianapolis, IN), weighing 290-295g at the beginning of the research study, were housed in plastic cages (22" L x 13" W x 8" H) with bar metal tops. Fine wood chips were placed down for bedding in the cage, and food and water were available *ad libitum*. The room in which the cages were kept was maintained at a temperature range of 21-24°C, and were kept on a 13-11 reversed light cycle (lights off between 11:00 a.m. and 12:00 a.m.). All subjects were originally housed in pairs. Post-surgery, subjects were housed individually to allow safe and proper healing of implanted cannulae. All procedures for this model were approved by the Institutional Animal Care and Use Committee of Wabash College.

Surgery

Stereotaxic surgery was only conducted when rats were between the weights of 300-350g, to allow the best chance for successful cannula placement. For cannulation surgery, rats received an injection of Nembutal (sodium pentobarbital) (Sigma; Saint Louis, MO), at a dose of 70 mg/kg. Once a surgical plane of anesthesia was achieved, the top of the head was shaved and prepped with an antibacterial solution prior to surgery. Unilateral cannulations were performed using a stereotaxic device to ensure accurate placement of the cannula. The cannula was aimed at 2 mm above the shell of the NA (van Erp and Miczek, 2000), using the relevant diagrams from The Atlas of Paxinos and Watson (2007). Once inserted into the brain, the cannula was held in place with jeweler's screws placed into the skull and secured with dental acrylic (Yates Motloid; Chicago, IL). Topical antibiotics (Neosporin, CVS; Woonsocket, RI) were given immediately following surgery, and rats were treated with acetaminophen (5 mL Children's Tylenol) (Johnson and Johnson; New Brunswick, NJ) to manage pain for 48 hours post-surgery (via home cage water bottle). All rats were given a one-week period for recovery, which was assessed based upon observed movement and other indications, such as weight and eating behavior. All rats recovered by seven days post-surgery.

Euthanasia

All rats were euthanized with a lethal dose of sodium pentobarbital (75 mg intraperitoneally) (Sigma; Saint Louis, MO) after completion of the study. Subsequently, all rats underwent perfusion for histological confirmation of cannulae placements.

Histology

Brain histology was conducted in order to determine the location of the cannulae. Upon euthanasia, brains were removed and immersed

in 30% sucrose, 10% buffered formaldehyde solution (Azer Scientific; Morgantown, PA) for fixation (Aubele and Kritzer, 2011). Once the brains sank, they were adhered to a dissection disk using tissue-freezing-medium (TFM) (Sigma, Saint Louis). The brain was then fixed to the cryostat and rapidly frozen to -25°C. Sections of the coronal plan were taken at 70μm. For all animal subjects, a 1 in 3 series of sections taken from the NA was collected, slide mounted, and Nissl stained with cresyl violet (Sigma; Saint Louis, MO) (Aubele and Kritzer, 2011).

Experimental Paradigm
Infusions.

Following recovery from surgery, the study was conducted over the course of one week. Rats were selected at random to be placed into two groups: those that received saline (0.9%) and those that received the 5-HT_{1B} agonist sumatriptan (300 μg/kg) (Sigma; Saint Louis, MO) dissolved into 0.9% saline (Reuter et al., 2004). Infusions occurred every day at 4:00 pm, for a total of 5 infusions, before the confrontation period at the end of the week. The final infusion was given to each pair of rats just before the behavioral analysis occurred. The extent of diffusion of sumatriptan into the NA shell and surrounding areas was not specifically identified; however, use of this infusion paradigm in the past (verified with dye staining) has indicated that diffusion of drug usually extends in a spherical shape around the tip of the cannula from 0.05-0.2mm (Lorrain et al., 1997)

Testing of Aggressive Behavior

For aggression testing, an open field chamber was adapted as a neutral ground (32”L x 32”W x 11”H). The field was filled with the same type of bedding used in the housing of the rats. Rats were tested in pairs: one saline rat versus one sumatriptan-infused rat. Only one session for aggressive confrontation occurred for each pair of rats, lasting a total of thirty minutes each. Aggressive offensive behaviors were as follows: attack latency, rearing, lateral threat, upright posturing, clinch attack, keep down (pinning), chasing, and biting (Koolhaas et al., 2013; see below for operational definitions of behavior). All rats were to be separated from confrontation either 5 minutes after the first bite to either rat, after 20 total bites, or, if no biting occurred, when the thirty-minute session was completed (van Erp and Miczek, 2000). Additionally, rats were to be separated upon the presence of bloodshed or the occurrence of a serious injury. No serious injury or biting occurred during aggressive bouts, thus all rats completed their 30-minute sessions.

Behavioral Analysis

Aggressive behaviors were scored and measured based upon the operational definitions for aggression as defined by Koolhaas et al. (2013). *Attack latency* was defined as the time between the introduction of the subjects and the first clinch attack. *Clinch attacking* was observed as an inter-locking and tumbling between the two rats. *Rearing* was scored as an upright posturing from one rat toward another, with the arms slightly out and toward the opposing rat. *Chasing* was scored as one rat following another rat at a quickened pace, attempting to catch the flank of the opposing rat. *Upright posturing* was scored as the two rats interlocked and leaning upon one another, while standing on the hind legs. *Lateral threats* were defined as the offensive (aggressing) rat exposing the side and flank of their body toward the other rat, with the raising of the back fur resulting in an increased look of the body size. *Keep down*, also known as *pinning*, looks very similar to the previously mentioned clinch attack, except that the rats are still. Thus, *keep down/pinning* was observed as the offensive rat turning the non-aggressing rat over to their back, while holding the rat down for a given time. Finally, *biting* was recorded as an offensive aggressive behavior, in which the aggressive rat placed the mouth around any part of the other rat and closed the jaw. Biting can result in minor to serious injury and was observed closely in order to maintain the safety of all subjects involved in the study. All forms of aggression were recorded (via video) and played back for scoring in accordance with the behaviors described above.

Data Analysis

The data for each aforementioned behavior was scored for each subject separately based upon the recorded video. The observer of

the video (NB) was blinded to the group (saline vs. sumatriptan) of each animal during behavior scoring. This procedural "blinding" was obtained by randomly numbering and marking the tails of the rats. The random numbering of rats came from selecting them for either the saline or sumatriptan groups before infusion began. Then, during behavioral testing, one rat participant in each bout (saline or sumatriptan) obtained at random a dark mark on the tail to allow for differential identification upon rescoring. Video file names and data sheets did not identify rat numbers. Thus, the observer was blinded to the rat's number and group during video re-scoring and based aggressive behavior scoring upon the tail markings ("dark tail" or "light tail") during the rescoring session. Rat numbers were only applied after the final re-scoring to identify the grouping of the rat. For each aggressive behavior, scoring was done separately. A *t*-test for two independent samples was used to test for differences among groups for each aggressive behavior. The alpha level at which significance was determined was .05.

Results

Cannula Placement Confirmation

Cannula placement verification was conducted and supported by the supervision of two observers (the two authors). Post-histology, seven of the eight cannulae were confirmed to be located within the lateral shell of the NA. The cannula placement of the eighth animal was carefully scrutinized and later confirmed to be on the border of the lateral NA. These confirmations were based upon observations made via light microscope, in correspondence to images and diagrams provided by Paxinos and Watson (2007). A representative placement may be seen in Figure 1.

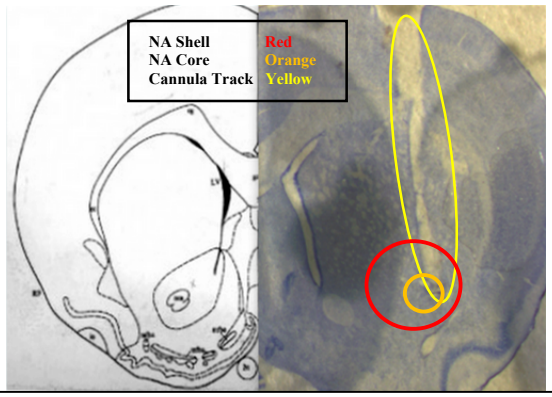

Figure 1: Above is a histological representation of the cannula placement for rats involved in this study. The coronal section on the left is reproduced from Paxinos and Watson (2007). The shell of the NA is represented by the large dotted circular shape on the lower portion of the image to the left, while the smaller circular shape within represents the core of the NA. The image on the right shows placement within the lateral shell of the accumbens.

Analysis

The results of the independent sample *t*-test for chasing indicated a significant effect of sumatriptan in the NA on chasing behavior, such that it was higher in rats receiving sumatriptan (M=8, SD=4.24; Saline M=2.5, SD=1.29); t (6) = -2.48, *p*=.048 (see Fig. 2).

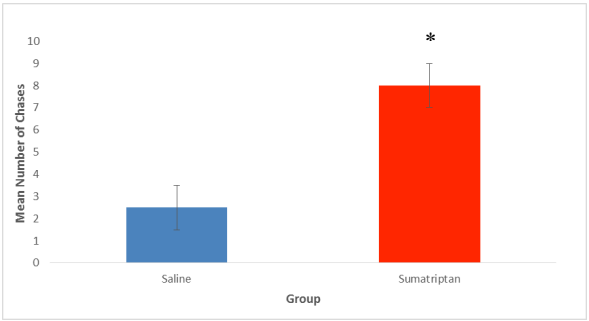

Figure 2: The above figure represents the mean number of chasing bouts initiated by saline (blue bar) and sumatriptan (red bar) rat groups. Capped lines indicate the standard error of the mean (SEM)

Furthermore, a significant effect of sumatriptan in the NA was seen for the initiation of clinch attacks, such that rats receiving sumatriptan initiated all clinch attacks; saline (SD=0), sumatriptan (SD=2.08); t (6) = -3.36, p= .015 (see Fig. 3).

Figure 3: The above figure represents the mean number of clinch attack bouts initiated by saline and sumatriptan (red bar) rat groups. All clinch attacks were initiated by rats receiving sumatriptan.

For all other behaviors coded, including lateral threat, upright posturing, rearing, and pinning, no significant differences between the sumatriptan and saline groups were found. Instances of biting were not seen in either group. For means, totals of each group's behaviors, standard errors, and level of significance see Table 1.

	Means		Totals of Behavior		Standard Error		Level of Sig.
	Saline	Sumatriptan	Saline	Sumatriptan	Saline	Sumatriptan	
Chasing	2.5	8	10	32	0.645	2.121	0.048*
Clinch Attack	0	3.5	0	14	0	1.041	0.015*
Lateral Threat	2.75	1.75	11	7	0.479	1.031	0.413
Pinning	1.50	1.25	9	5	0.854	0.479	0.852
Upright Posture	.50	.25	2	1	0.289	0.25	0.537
Rearing	.25	.75	1	3	0.25	0.479	0.39
Biting	0	0	0	0	0	0	N/A

Table 1: The table above provides the statistical representation of behavioral means, totals, standard error for each behavior, and the p-value for each behavior. * Indicates significant data at the $p<.05$ level.

Discussion

In summary, the results indicate that sumatriptan rats increased chasing and clinch attack behaviors, but not other aggressive behaviors. The data suggests an association between sumatriptan in the shell of the NA and particular aggressive behaviors, namely chasing and clinch-attacking. It appears that 5-HT_{1B} receptors in the NA may at least partially modulate aggressive behaviors in rats, and, given that aggression was increased and not decreased in those rats receiving sumatriptan, this modulation may be via postsynaptic effects on 5-HT_{1B} receptors, as we hypothesized. Therefore, it appears that the increase in serotonin seen by Miczek et al (2000) may be functionally related to increased aggression in rodents.

Intriguingly, the two behaviors affected by sumatriptan share the commonality of being in the beginning stages of confrontation; that is, rats will engage in these behaviors before others. They are arguably the most informative behaviors, given the purpose of this research: to investigate the basic neural cause (if any) for naturally aggressive behavior. Correspondingly, the beginning or initiation stages of aggressive behaviors may suggest more basic levels of aggression, such as threat (Tremblay et al., 2004). The current experimental approach of a neutral-cage setting enabled any priming external factors for aggression to be reduced, if not eliminated. Our findings suggest that this neutral-cage model may be useful for investigating initiation or initial-contact (chasing and clinch attack) aggressive behaviors.

These behaviors importantly relate to the overall escalation of aggression, as argued by Koolhaas et al., (2013). However, it can be argued that these behaviors may be affected by external or circumstantial factors, such as the size of the rat, or interpreted as being defensive (e.g., rearing), versus offensive aggressive behaviors. As Blanchard and colleagues' (2003) research suggests, offensive aggression involves responding to being challenged over adaptively important resources, whereas defensive aggression is attacking in order to defend the subject's own bodily integrity. Additionally, the bodily location in which attacks occur suggests the type of attacks carried out. Attacks to the back and flanks in altercations involving rats suggest offensive aggression, while attacks to the snout and head would be considered defensive aggression (Blanchard et al., 2003). Further research with this model should explore and analyze the bodily locations in which aggressive behaviors are conducted. This could lead to further categorization of the aggressive behaviors carried out in this experiment and future studies. Future research focused on

categorizing offensive vs. defensive aggressive behavior, along with more thoroughly defining provocation/instigation behavior is necessary. Moreover, in further developing such a definition for these initial aggressive behaviors, a correlation between chasing and clinch attack should also be investigated. In conducting an analysis upon these aggressive behaviors, they were analyzed independently. Future research may identify that one of these behaviors may be dependent upon another. Thus, a clinch attack may occur after a number of chases occurred or vice-versa. In the current study, this was a point of interest, but it is something that would need to be analyzed more thoroughly with a larger sample of rats.

While the current experiment introduced a novel method for testing and analyzing aggressive behaviors in rats, there were some limiting factors, such as the small sample size for each group of rats. Additionally, the amount of available subjects limited the testing factors of this experiment. Other limitations include not specifically testing the diffusion of the drug from the cannula tip; thus, drug could have infused into the core of the NA. Furthermore, the current experimental design only included one predetermined dose of sumatriptan. Additional experiments should investigate different dosages of the drug and its effectiveness. Future experiments will pair saline vs. saline and sumatriptan vs. sumatriptan rats to further understand the propensity of animals to be aggressive without any behavioral or pharmacological priming, or when both animals are pharmacologically primed. Future research calls for the dialysis of levels of 5-HT in the NA during the confrontation period after the final infusion of sumatriptan. This would permit a further understanding of the levels of 5-HT involved in the NA during aggressive instances while in a neutral setting and confirm the expected effects of sumatriptan on NA 5-HT levels.

The root purpose for this experiment was an investigation behind a potential neural cause or predisposition for aggressive behavior. While extensive future research and investigation is necessary, this current study opens the door to research involving aggression and motivation in criminal behavior. With aggression being a key component of certain criminal behaviors, it is of the utmost importance that this research be carried out further to identify a potential source for these behaviors.

In conclusion, the outcomes of our research suggest and support the use and further exploration of a neutral paradigm for testing aggression in a rodent model as well as introducing a potential contributing mechanism for unprimed aggression via activation of postsynaptic 5-HT_{1B} receptors in the lateral shell of the NA.

Acknowledgements

The authors would like to thank Dr. Neil Schmitzer-Torbert and Wabash College for providing the necessary means to conduct this research, including laboratory space and monetary support for purchasing rats.

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References

Aronson E (2007) The social animal. New York: Worth Publishers.

Aubele T, Kritzer M (2011) Gonadectomy and hormone replacement affects in vivo basal extracellular dopamine levels in the prefrontal cortex but not motor cortex of adult male rats, Cereb Cortex 21:222-32.

Blanchard R, Wall P, Blanchard D (2003) Problems in the study of rodent aggression, Hormones and Behavior 44:161-70.

Berman ME, Tracy JI, Coccaro EF (1997) The serotonin hypothesis of aggression revisited, Clinical Psychology Review 17:651-65.

Cologer-Clifford A, Simon NG, Lu SF, Smoluk SA (1997) Serotonin agonist-induced decreases in intermale aggression are dependent on brain region and receptor subtype, Pharmacology Biochemistry and Behavior 58:425-30.

de Almeida R., Ferrari P, Parmigiani S, Miczek K (2005) Escalated aggressive behavior: Dopamine, 5-HT, and GABA, European Journal of Pharmacology 52:51-64.

de Almieda RM, Lucion AB (1997) 8-OH-DPAT in the median raphe, dorsal periaqueductal gray and corticomedial amygdala nucleus decreases, but in the medial septal area it can increase maternal aggressive behavior in rats, Psychopharmacology (Berl) 134:392-400.

de Almieda RM, Nikulina EM, Faccidomo S, Fish EW, Miczek KA (2001) Zolmitriptan-a 5-HT1B/D agonist, alcohol, and aggression in mice, Psychopharmacology (Berl) 157:131-41.

de Boer SF, Koolhaas JM (2005) 5-HT1A and 5-HT1B receptor agonists and aggression: A pharmacological challenge of the serotonin deficiency hypothesis, European Journal of Pharmacology 526:125-39.

Ferris CF, Stolberg T, Deville Y (1999) Serotonin regulation of aggressive behavior in male golden hamsters (Mesocricetus auratus), Behavioral Neuroscience 113:804-15.

Fletcher P, Korth K (1999) Activation of 5-HT1B receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward, Psychopharmacology 142:165-74.

Fletcher PJ, Korth KM (1999) RU-24969 disrupts d-amphetamine self-administration and responding for conditioned reward via stimulation of 5-HT1B receptors, Behavioral Pharmacology 10:183-93.

Furay AR, McDevitt RA, Miczek KA, Neumaier JF (2011) 5-HT1B mRNA expression after chronic social stress, Behavioral Brain Research 224:350-7.

Groenik L, van Bogaert M., van der Guten J, Oosting S, Olivier B (2003) 5-HT1A receptor and 5-HT1B receptor knockout mice in stress and anxiety paradigms, Behavioural Pharmacology 14:369-83.

Kohlert JG, Mangan BP, Kodra C, Drako L, Long E, Simpson H (2012) Decreased aggressive and locomotor behaviors in Betta splendens after exposure to fluoxetine, Psychological Reports 110:51-62.

Koolhaas J, Coppens C, de Boer S, Buwalda B, Meerlo P, Timmermans P (2013) The resident-intruder paradigm: A standardized test for aggression, J Vis Exp 77*:* 43-67.

Kravitz EA, Huber R (2003) Aggression in invertebrates, Current Opinions in Neurobiology 13:736-43.

Lorrain DS, Matuszewich L, Friedman RD, Hull EM (1997) Extracellular serotonin in the lateral hypothalamic area is increased during the postejaculatory interval and impairs copulation in male rats, Neuroscience 23:9361-6.

Miczek KA, Hussain S, Faccidomo S (1998) Alcohol-heightened aggression in mice: Attenuation by 5-HT1A receptor agonists, Psychopharmacology (Berl) 139:160-68.

Mongillo DL, Kosyachkova EA, Nguyen TM, Holmes MM (2014) Differential effects of chronic fluoxetine on the behavior of dominant and subordinate naked mole-rats, Behavioral Brain Research 258:119-26.

Nair SG, Furay AR, Liu Y, Neumaier JF (2013) Differential effect of virual overexpression of nucleus accumbens shell 5-HT1B receptors on stress- and cocaine priming-induced reinstatement of cocaine seeking, Pharmacology Biochemistry and Behavior 112:89-95.

Olivier B, van Oorschot R (2005) 5HT-1B receptors and aggression: a review, European Journal of Pharmacology 526:207-17.

Olivier B, Young L (2002) Neuropsychopharmacology, American College of Neuropsychopharmacology 5*:*1699-708.

Paxinos G, Watson C (2007) The rat brain: In stereotaxic coordinates*,* Stereotaxic Reference Section 1:6.

Pineyro G, Blier P (1999) Autoregulation of serotonin neurons: Role in antidepressant drug action, Pharmacological Reviews 51:533-91.

Raleigh MJ, McGuire MT, Brammer GL, Yuwiler A (1984) Social and environmental influences on blood serotonin concentrations in monkeys, General Psychiatry 4:405-10.

Reuter U, Salomone S, Ickenstein G, Waeber C (2004) Effects of chronic sumatriptan and zolmitriptan treatment on 5-HT1 receptor

expression and function in rats, Cephalalgia 24:398-407.

Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot MC, Hen R (1994) Enhanced aggressive behavior in mice lacking 5-HT1B receptor, Science 265:1875-8.

Simon NG, Cologer-Clifford A, Lu SF, McKenna SE, Hu S (1998) Testosterone and its metabolites modulate 5HT1A and 5HT1B agonist effects on intermale aggression, Neuroscience and Behavioral Reviews 23:325-36.

Spoont M (1992) Modulatory role of serotonin in neural information processing: Implications for human psychopathology, Psychological Bulletin 112:330-50.

Summers CH, Korzan WJ, Lukkes JL, Watt MJ, Forster GL, Overli O, Hoglund E, Larson ET, Ronan PJ, Matter JM, Summers TR, Renner KJ, Greenberg N (2005) Comparing regional activity before and during social interaction, Physiological and Biochemical Zoology 5:679-94.

Tidey J, Miczek K (1996) Social defeat stress selectively alters mesocorticolimbic dopamine release: An in vivo microdialysis study, Brain Research 721:140-9.

Tremblay R, Nagin D, Seguin J, Zoccolillo M, Zelazo P, Boivin M (2007) Physical aggression during early childhood: Trajectories and predictors, Pediatrics 4:43-50.

van Erp A, Miczek K (2000) Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats, The Journal of Neuroscience 20:9320-5.