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RESEARCH ARTICLE | *Higher Neural Functions and Behavior*

Social experience alters socially induced serotonergic fluctuations in the inferior colliculus

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Keesom SM, Sloss BG, Erbowor-Becksen Z, Hurley LM. Social experience alters socially induced serotonergic fluctuations in the inferior colliculus. *J Neurophysiol* 118: 3230–3241, 2017. First published August 30, 2017; doi:10.1152/jn.00431.2017.—Past social experience and current social context shape the responses of animals to social signals. The serotonergic system is one potential mechanism by which both experiential and contextual factors could be conveyed to sensory systems, such as the auditory system, for multiple reasons. *1*) Many features of the serotonergic system are sensitive to social experience. *2*) Elevations in serotonergic activity are triggered by social partners, and variations in socially triggered serotonergic responses reflect behavioral differences among social encounters. *3*) Serotonin is an auditory neuromodulator, altering how auditory neurons respond to sounds including conspecific vocalizations. In this study, we tested how social experience influences the socially triggered serotonergic response in the inferior colliculus, an auditory midbrain region with an important role in vocalization processing. We used carbon fiber voltammetry to measure serotonin during social interactions of male mice (*Mus musculus*) from different social backgrounds: 4 weeks of grouped or individual housing. When paired with an unfamiliar male, both group-housed and individually housed males demonstrated elevations in serotonin; however, individually housed males exhibited socially triggered serotonergic responses with delayed time courses compared with the group-housed males. Furthermore, group-housed males displayed previously described correlations between the socially triggered serotonergic response and behaviors such as social investigation. In contrast, individually housed males did not show these serotonin-behavior relationships. These results suggest that social experience gained via social housing may shape the ability of the central serotonergic system to encode social context in sensory regions.

NEW & NOTEWORTHY We demonstrate that past social experience influences the fidelity with which the serotonergic system represents social context in an auditory region. Social experience altered the time course of socially triggered serotonergic responses and changed how the serotonergic system reflects behavioral variations among social encounters of the same context. These findings are significant to the study of communication, suggesting that centralized neuromodulatory systems potentially convey integrated information regarding past experience and current context to primary sensory regions.

serotonin; inferior colliculus; auditory; social isolation; social competence

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INTRODUCTION

The ability of an animal to fine-tune its behavioral and corresponding physiological responses on the basis of current social information is referred to as "social competence" (Oliveira 2009). Social animals live in a dynamic world, where the progression and outcome of social encounters can vary depending on a multitude of factors, including past experience (Summers 2002). An animal's ability to flexibly grade its physiological responses according to these variations among contexts is potentially adaptive, resulting in the most appropriate behavioral response to a given situation (Taff and Vitousek 2016). Sensory systems play a key role in the coordination among external events, internal physiology, and behavioral responses and on the influence of experience on these associations.

There is ample evidence that sensory systems are sensitive to social experience. For example, starlings that are raised with conspecific adults have a higher proportion of selective auditory cells in an auditory region, field L, compared with starlings raised with broadcast song but no direct social interaction (George and Cousillas 2013). A different type of experience, maternal experience, alters how neurons in the auditory cortex of female mice (*Mus musculus*) respond to pup isolation calls. Females with pup experience demonstrate faster and larger evoked neuronal responses, along with enhanced entrainment, to pup vocalizations (Liu and Schreiner 2007; Liu et al. 2006; Miranda and Liu 2009). Experience not only influences the absolute auditory response but also shapes how auditory input is integrated with nonauditory contextual information. In starlings, early social experience influences how visual input modulates auditory responses. Visual cues of familiar adults alter auditory responses in starlings raised with adults, whereas starlings raised without adults do not demonstrate a multisensory representation of familiar stimuli (George et al. 2011, 2012). In the case of female mice (*M. musculus*), maternal experience alters how olfactory cues (pup odors) influence auditory responses to pup calls: pup odors cause significantly more modulation in auditory response of cortical neurons in females with pup experience compared with naive females (Cohen et al. 2011). Taken together, these studies demonstrate that social experience is a significant modulator of receivers' responses to communication signals at the level of primary auditory regions. However, the neural mechanisms that promote the integration of experience and current context with sensory processing need more investigation.

The central serotonergic system, situated mainly in the raphé nuclei, is a candidate mechanism by which information regarding both social history and current social context could be integrated with sensory processing (Petersen and Hurley 2017). The raphé nuclei are targeted by a diverse set of brain regions that relay different aspects of social information: primary sensory regions and higher order social centers, including nodes of the vertebrate social behavior network and regions important for processing reward (Goodson 2005; Pollak Dorocic et al. 2014; Vertes and Linley 2008). The array of synaptic input to the raphé nuclei suggests that the serotonergic system has the potential to integrate and then redistribute the social information it receives. Furthermore, serotonergic neurons respond to a variety of stimuli, including the presence of social partners (Li et al. 2016). Serotonergic fibers innervate the majority of brain regions, including primary sensory regions, and serotonin that is endogenously released or exogenously applied to sensory regions modulates sensory responses, including olfactory (Lottem et al. 2016), electrosensory (Deemyad et al. 2013), and auditory processing (Hurley and Sullivan 2012). Finally, serotonergic activity within sensory regions such as the auditory midbrain is graded with the intensity of behavioral interaction in subject males interacting with either males or females (Hall et al. 2011; Keesom and Hurley 2016).

Mice provide a tractable model system in which to investigate how social experience influences the neurophysiology of receivers for several reasons. First, mice are susceptible to a methodologically simple manipulation of social experience (grouped vs. individual housing), and this manipulation reliably alters the behavioral and neurophysiological phenotypes of these mice (Fone and Porkess 2008; Lukkes et al. 2009). Second, mice engage in complex social interactions that necessitate the exchange of signals across multiple modalities, with chemical and acoustic signals being particularly important (Arakawa et al. 2008; Asaba et al. 2014; Portfors 2007). Furthermore, the use of these signals is altered by social experience (Chabout et al. 2012; Keesom et al. 2017). In the inferior colliculus (IC), a mammalian auditory midbrain nucleus, endogenous fluctuations in serotonergic activity during social encounters between mice correlate with behavior (Hall et al. 2011; Hanson and Hurley 2014; Keesom and Hurley 2016). This suggests that auditory neuromodulation by serotonin could depend on features of context, because serotonin regulates how IC neurons respond to vocalizations (Hurley and Pollak 2005). We can therefore simultaneously investigate how social experience changes vocal output and receiver physiology by investigating socially induced serotonergic activity in an auditory region of mice during behavioral interactions.

Thus in this study we investigated how early-life social experience affects socially induced serotonergic activity by housing male mice either in social groups or as individuals for 4 wk. Because serotonergic activity is greater in the second of two social encounters separated by 5–7 days (Hall et al. 2011), we predicted that socially induced elevations in serotonergic activity would be larger in group-housed males compared with individually housed males. Previous work has also shown that serotonergic activity in the inferior colliculus during social interactions reflects variation in context, via correlation with both social and nonsocial behavior. For instance, serotonergic activity in resident male mice is positively related to social investigation directed at intruder males (Hall et al. 2011). Thus we also investigated whether social experience acquired via group housing affected relationships between serotonergic activity and behavioral variation among social encounters.

MATERIALS AND METHODS

Animals and social housing treatment. All procedures were approved by the Bloomington Institutional Animal Care and Use Committee (Indiana University). Male CBA/J laboratory mice (*M. musculus*) from different litters (Jackson Laboratory, Bar Harbor, ME) arrived in our laboratory immediately following weaning (3 wk old) and were placed into social treatments for 4 wk. Subject mice were housed either individually or in groups of three; intruder mice were housed individually. A total of five cohorts were used ($n = 45$ mice); each cohort consisted of nine non-kin mice, comprising three individually housed subject mice, three group-housed subject mice, and three individually housed intruder mice per cohort. Because of the challenging nature of recording physiological data with fragile electrodes from awake and behaving animals interacting with social partners, we were only able to obtain data from a subset of these mice. Therefore, 9 of 15 group-housed subject mice, 10 of 15 individually housed subject mice, and 15 individually housed intruder (stimulus) mice contributed to the study. All mice were housed in standard laboratory cages on a 14:10-h light-dark cycle and were provided with water and standard laboratory mouse chow ad libitum.

Socially housed mice were assessed for dominance on the day preceding cannulation surgeries by being placed in a chamber with a transparent tube linking two compartments. "Tube dominance" is a reliable measure of dominance for male mice, correlating across other tests of dominance and with many physiological variables (Howerton et al. 2008; Lindzey et al. 1961; Wang et al. 2011). Mice from the same home cage were assessed in pairs, in a round robin design. To begin the 5-min trial, the mice were positioned at opposite ends of the transparent tube and released simultaneously. The mouse that remained in the tube for the duration of the trial was designated as "dominant," and the mouse occupying one of the side chambers was designated as "subordinate." Group-housed mice exhibited a pattern of social ranking with one dominant male and two subordinates (Wang et al. 2011). Individually housed animals were allowed to interact with the tube and side compartments alone and largely remained in the tube. Between each trial, the tube and chamber were cleaned with laboratory detergent and wiped down with 70% ethanol. After dominance assessment, all mice were individually housed in new cages for the remainder of the study.

Surgery. Aseptic surgeries were performed to permanently mount voltammetric headstages on the skull overlying both IC. For each cohort, the three individually housed mice underwent surgery on one day, and the three group-housed mice underwent surgery on a different day. Before the start of surgery, the mouse was administered a dose of meloxicam analgesic (1 mg/kg; subcutaneous) and anesthetized via an intraperitoneal injection of ketamine (100 mg/kg), xylazine (5 mg/kg), and acepromazine (2 mg/kg). Depilatory cream was used to remove the hair from the top of the head. Following disinfection of the skin with Betadine and 70% ethanol, the mouse was positioned in a stereotaxic device and an incision was made to expose the skull surface above the IC. Bregma and lambda were placed in the horizontal plane, and 1.5-mm holes were drilled over both IC (1.1 mm posterior, 1.6 mm lateral from lambda). Dental cement was used to affix the custom-built Teflon hubs to two stainless steel bone screws (one rostral and one caudal to the hubs), and the hubs were plugged with Teflon screws. The skin around the surgical site was secured and closed with Vetbond (3M) adhesive, completing the surgery. Mice recovered for at least 5 days before voltammetric recording sessions. Thus mice were 8–9 wk old during voltammetric recording sessions.

Carbon fiber voltammetry. Carbon fiber electrodes (CFEs) were constructed from pulled glass capillary tubes. The glass tip was broken to an 11-μm interior diameter, and a carbon fiber (Thornell

P25; Cytec Industries, West Paterson, NJ) was inserted from the back end until it protruded 100–150 μm from the tip. The carbon fiber was secured in place with epoxy resin (Miller-Stephenson Chemical, Danbury, CT) and soldered to a tinned copper wire with low-melting bismuth alloy.

All CFEs were electrically and chemically pretreated to increase sensitivity to and selectivity for serotonin, as described previously (Hall et al. 2010; Keesom and Hurley 2016). CFEs were electrically pretreated by suspension in phosphate-citrate buffer and application of the following protocol (vs. a Ag-AgCl reference electrode and a calomel auxiliary electrode), using an eCorder controlled by Chart software (eDAQ, Denistone East, NSW, Australia): 70 Hz, 0.3 V triangle waveform for 30 s, 1.5 V for 10 s, −0.5 V for 5 s, and 1.5 V for 8 s. This pretreatment separated the oxidation potentials of ascorbic acid, catecholamines, and serotonin into three discernible peaks on a plot of applied voltage vs. the first derivative of the current. Subsequently, CFEs were chemically pretreated by dip-coating with Nafion ion-exchange resin (5% Nafion solution; Sigma-Aldrich, St. Louis, MO). These electrical and chemical treatments resulted in CFEs that were sensitive to and selective for serotonin (Hall et al. 2010; Keesom and Hurley 2016). In vitro performance of all CFEs was verified in a solution of ascorbic acid, 3,4-dihydroxyphenylacetic acid (DOPAC), and serotonin dissolved in phosphate-citrate buffer. CFEs were used during in vivo recording sessions only if the third oxidative peak (serotonin) was greater than *peaks 1* and *2* (ascorbic acid and DOPAC) on a plot of the first derivative of current measured after applying the voltage waveform vs. time. This indicated a functional Nafion coating.

To measure serotonin for both in vitro pretests and in vivo recording sessions, a bipotentiostat and eCorder controlled by EChem software (eDAQ) recorded the current produced when a 1-min cyclic staircase voltage waveform (range −300 to +600 mV with 10-mV steps at 30 mV/s^2) was applied to the CFE against a Ag-AgCl reference electrode, with 5 min between each waveform. This resulted in one measurement of serotonin every 6 min, representing a compromise between maximizing the number of measurements during recording sessions and use-dependent biofouling of the carbon fiber surface, a characteristic property of CFEs (Singh et al. 2011). For in vivo recording sessions, we measured serotonergic oxidation as the amplitude of the third oxidative peak (~380 mV) relative to the adjacent troughs. Measurements of serotonergic oxidation at each time point were normalized as percent change relative to the average of the four baseline measurements made immediately before the introduction of the intruder, to account for variability among individual experiments that could reflect variation among individual animals or variation in electrode sensitivity. We refer to these normalized measurements as "serotonergic activity" as in Keesom and Hurley (2016).

Voltammetry recording sessions. On the day of a recording session, the subject mouse was briefly anesthetized with isoflurane fumes and administered meloxicam (1 mg/kg Metacam; subcutaneous) and 60% of the surgical dose of ketamine-xylazine (intraperitoneal). A Teflon microdrive loaded with a pretested CFE was secured in one of the hubs, and a microdrive containing the Ag-AgCl reference electrode was fitted in the other. The mouse was then transported in its home cage to the experiment room, and the CFE was lowered into the IC. Voltammetric recording began immediately to assess the performance of the electrode. Two hours after recovery from anesthesia, 4 baseline measurements were recorded at 6-min intervals while the subject mouse occupied the empty cage. Immediately thereafter, a male intruder mouse was introduced to the cage. The subject and intruder interacted freely for 30 min, during which 5 measurements of serotonergic activity were made at 6-min intervals ("intruder" measurements). The intruder was removed after the fifth measurement, and three "post-intruder" measurements were made with the resident subject mouse alone in the cage. Recording sessions were only used if 2 of 4 baseline measurements were discernable from movement-related noise and if 2 of 5 "intruder" measurements were usable.

For 5 of 9 group-housed mice and 4 of 10 individually housed mice, serotonergic activity was also measured during a second recording session with a novel intruder mouse (in the IC contralateral the side used during the first recording session). The reduction in sample size for the second recording session was due to technical issues such as a reduction in signal-to-noise ratio, electrode breakage, or short-circuiting. Mice were allowed at least 5 days of recovery between recording sessions. At the end of the second recording session, a current was passed through the carbon fiber by a constant current lesion maker (Grass Instruments, Quincy, MA). Perfused brains were sliced in the coronal plane, and all lesions were confirmed to be in the IC.

Audio and video analysis of behavior. Resident-intruder encounters were recorded with a charge-coupled device video camera (30 frames/s; Q-See 4-channel DVR PCI video capture card, SuperDVR software; Digital Peripheral Solutions) and condenser microphone (CM16/CMPA; Avisoft Bioacoustics; 200-kHz maximum range) with a sound card (UltraSoundGate 116 Hb; Avisoft Bioacoustics; 250-kHz sample rate). All vocal and nonvocal behavioral analyses were conducted blind to treatment group. Vocalization analysis was performed using sound spectrograms generated by Avisoft SASlab Pro software (512 fast-Fourier transform length and Hamming-style window with 50% overlap; Avisoft Bioacoustics). Male mice emit two broad classes of calls when encountering other males: broadband squeaks (2–100 kHz) and narrowband, ultrasonic vocalizations (USVs; >20 kHz). The total number of squeaks and USVs were counted for each resident-intruder interaction. USVs were further categorized into two types: 50-kHz USVs (lowest frequency near 50 kHz, with a harmonic frequency at 100 kHz) and 70-kHz USVs (lowest frequency near 70 kHz, no visible upper harmonic). These distinctions were made because mice can distinguish between USVs with harmonic elements and USVs without harmonic elements (Neilans et al. 2014). Additionally, USVs containing harmonic components are behaviorally significant, being related to mounting behavior directed toward both males (Keesom et al. 2017) and females (Hanson and Hurley 2012).

Nonvocal behaviors were analyzed via video recording, using ODLog (Macropod Software, Eden Prairie, MN). The following social behaviors were measured and defined as follows: "resident anogenital investigation" as the subject resident mouse's nose in contact with the region around the base of the intruder mouse's tail; "intruder anogenital investigation" as the intruder mouse's nose in contact with the region around the base of the resident's tail; "nose-to-nose investigation" as the nose of one mouse in contact with the other mouse's facial region; "mounting" as the intruder mouse's front paws on the back of the other mouse (mounting was not performed by resident subject mice); and "bucking" as the subject mouse dislodging the intruder mouse from mounting by kicking or lunging away from the intruder. As a measure of nonsocial behavior, we scored "inactivity" as the resident mouse remaining stationary and not interacting with the intruder in any way.

Statistical analysis. Statistical analyses were conducted using SPSS version 23.0 (IBM). To test whether group-housed and individually housed mice differed in the behaviors displayed during social interaction, we used Welch's unequal variance *t*-tests (Ruxton 2006). USVs, the percentage of 50-kHz USVs per total USVs, the number of squeaks, and the number of bucks were square-root transformed to attain normality. We used Spearman rank correlations to test for relationships between mounting and bucking in the group-housed and individually housed treatment groups, and we used Fisher's *r*-to-*z* transformation (Weaver and Wuensch 2013) to test whether the correlation coefficients were significantly different. We used Mann-Whitney *U*-tests to assess whether dominant ($n = 5$) and subordinate ($n = 4$) mice differed in vocal and nonvocal behaviors, because of the small sample sizes. All tests were controlled for the false discovery

rate using the Benjamini-Hochberg method (Benjamini and Hochberg 1995).

To account for partial repeated measures resulting from noise due to movement or electrode breakage, a linear mixed model was used to test for effects of social housing treatment, recording session number (first vs. second), and time on serotonergic activity at each of the eight time points (5 intruder and 3 post-intruder measurements). To test whether social experience influenced the time point with maximal serotonergic activity, we used a two-way ANOVA with social housing treatment and recording session number (first vs. second) as main factors. Finally, to test whether social dominance status of group-housed mice affected serotonergic activity, we used a linear mixed model including only the group-housed mice, with dominance status and time as the main factors. Although there was a statistical difference with respect to dominance status in the number of USVs emitted during social interactions, with more USVs being emitted during interactions with subordinate mice (Mann-Whitney $U = 0$, $P = 0.014$), dominance status had no significant effect on the socially induced elevation in serotonergic activity (mixed-model $F = 0.001$, $P = 0.980$). Dominance status also had no significant effect on any of the other behaviors we measured. All post hoc pairwise comparisons were conducted using the Bonferroni method.

Spearman rank correlations were used to test for relationships between mean serotonergic activity and behaviors in the socially housed and individually housed treatment groups. Mean serotonergic activity for a single social encounter was calculated as the mean of the two measurements taken at 24 and 30 min after introduction of the intruder mouse, because serotonergic activity was significantly elevated over baseline at these time points for both group-housed and individually housed mice. For behaviors, either the total number of occurrences or the summed duration of the behavior over the 30-min resident-intruder encounter was used. To assess whether correlation coefficients for relationships between serotonin and behaviors were significantly different between group-housed and individually housed mice, we used Fisher's r-to-z transformation (Weaver and Wuensch 2013). The Benjamini-Hochberg method was used to control for false discovery rate (Benjamini and Hochberg 1995).

A summary of all statistical tests, variables tested, factors, and sample sizes is provided in Table 1.

RESULTS

Behavioral effects of social housing treatment and social dominance. Previous studies have shown that socially housed and individually housed mice differ in several aspects of social behavior (Fone and Porkess 2008). In the current study, we confirmed that our manipulation of social experience was effective by comparing behaviors displayed by mice from the two social housing treatments. Housing mice in social groups had a significant effect on the total number of USVs and the proportion of 50-kHz USVs, with a greater total number of USVs and higher proportion of 50-kHz USVs emitted during interactions with group-housed residents than with individually housed residents (total USVs: $t_{24.46} = 3.024$, $P = 0.006$; percent 50-kHz USVs: $t_{22.18} = 3.396$, $P = 0.003$; Fig. 1, *A* and *B*). Social housing also had a significant effect on broadband squeaks and bucking, with individually housed mice emitting significantly more squeaks and bucking significantly more than group-housed mice (squeaking: $t_{17.031} = -2.195$, $P = 0.042$; bucking: $t_{16.96} = -2.216$, $P = 0.041$; Fig. 1, *C* and *D*). This finding supports the efficacy of our social housing manipulation, because individually housed mice exhibit greater reactivity to both social and nonsocial stimuli (Cairns et al. 1985; Gariépy et al. 1995). Social housing had no effect on resident anogenital investigation ($t_{24.01} = 0.719$, $P = 0.479$), nose-to-nose investigation ($t_{25.95} = 0.46$, $P = 0.649$), intruder anogenital investigation ($t_{22.33} = -1.128$, $P = 0.271$), mounting ($t_{23.04} = -0.387$, $P = 0.702$), or inactivity ($t_{23.75} = 0.236$, $P = 0.816$).

Because male mice from both treatment groups were mounted equally but differed in the degree of bucking behavior (this behavior was typically displayed by residents in response to mounting attempts by intruders), we investigated whether social housing influenced the relationship between total number of mounts and total number of bucking incidences. For mice housed in social groups, mounting and bucking were not significantly correlated (Spearman correlation, $r_s = 0.407$, $n = 13$, $P = 0.149$; Fig. 2*A*). In contrast, for individually housed mice, there was a significant positive correlation between the number of times the intruder mounted the resident and the number of bucking incidences performed by the resident (Spearman correlation, $r_s = 0.771$, $n = 14$, $P = 0.001$; Fig. 2*B*). However, the correlation coefficients themselves were not significantly different from each other (Fisher's r-to-z test, $z = 0.437$, $P = 0.147$).

Social housing influenced the timing of socially induced serotonergic activity. As previously reported (Hall et al. 2011), there was a significant overall effect of time on serotonergic activity in male residents after introduction of a male intruder (mixed-model $F = 2.843$, $P = 0.002$), with average serotonergic activity increasing over time in males from both housing conditions (Fig. 3*A*). In contrast, there were no significant effects of housing condition (mixed-model $F = 0.004$, $P = 0.948$), first vs. second social intrusion (mixed-model $F =$

Table 1. *Statistical tests and corresponding sample sizes for analyses of effects of housing treatment and social dominance status.*

Statistical Test	Variable(s)	Factor(s)	Sample Size(s)
Welch's t-tests	Vocal and nonvocal behaviors	Housing treatment	SOC ($n = 13$); IND ($n = 14$)
Spearman rank correlations; Fisher's r-to-z transformation	Mounting and bucking	Housing treatment	SOC ($n = 13$); IND ($n = 14$)
Mann-Whitney U-tests	Vocal and nonvocal behaviors	Dominance status	DOM ($n = 5$); SUB ($n = 4$)
Linear mixed model	Serotonergic activity	Housing treatment, recording session number, and time	SOC ($n = 13$); IND ($n = 14$)
Linear mixed model	Serotonergic activity	Dominance status and time	DOM ($n = 5$); SUB ($n = 4$)
Spearman rank correlations; Fisher's r-to-z transformation	Serotonergic activity vs. behaviors	Housing treatment	SOC ($n = 13$); IND ($n = 14$)
Spearman rank correlation	50% Rise time in serotonergic activity; peak %50-kHz USVs	Housing treatment	SOC ($n = 10$); IND ($n = 11$)

SOC, socially housed; IND, individually housed; DOM, dominant; SUB, subordinate.

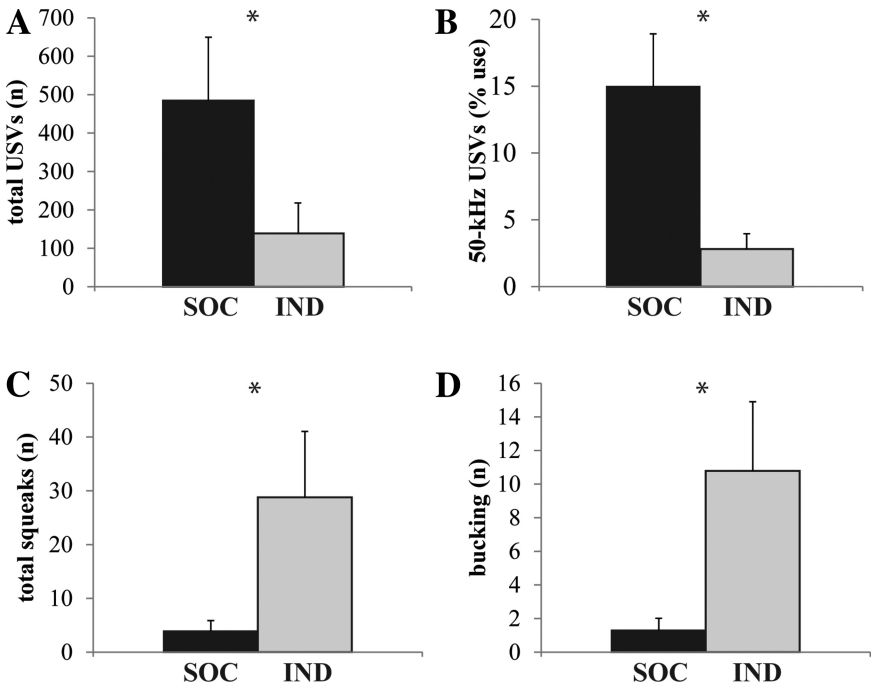

Fig. 1. Total number of ultrasonic vocalizations (USVs; *A*), proportional use of 50-kHz USVs per total USVs (*B*), total number of broadband squeaks (*C*), and total number of bucking incidences (*D*) exhibited during interactions between male resident mice of different social histories and an individually housed male intruder. SOC indicates resident subjects housed in social groups ($n = 9$); IND indicates resident subjects housed individually ($n = 10$). Bars represent means ± SE (Welch's unequal variance *t*-tests, $*P < 0.05$).

1.418, $P = 0.243$), or a housing × time interaction (mixed-model $F = 0.888$, $P = 0.553$) on serotonergic activity.

Although there was no difference in the amplitude of serotonergic activity between housing conditions, social experience influenced two features of the serotonergic trajectory. First, there was a difference between housing condition in the time point at which serotonergic activity was significantly elevated over baseline. Group-housed male mice ($n = 9$) demonstrated a significant elevation in serotonergic activity at 12 min ($P = 0.004$), whereas individually housed mice ($n = 10$) exhibited a delayed elevation, with serotonergic activity not being significantly elevated from baseline until 24 min after introduction of the intruder male ($P = 0.009$; Fig. 3*A*). Second, the peak time (time of maximal serotonergic activity) was significantly earlier for group-housed males compared with individually housed males (2-way ANOVA, $F_{1,13} = 4.693$, $P = 0.049$; Fig. 3*B*). There was no effect of recording session number (first vs. second) on peak time (2-way ANOVA, $F_{1,13} = 2.874$, $P = 0.114$). Thus group-housed males exhibited both a faster increase in serotonergic activity and an earlier peak.

We also measured the time course as the 50% rise time of the serotonergic trajectory for each individual male. These are depicted in Fig. 3*C* as normalized trajectories for the socially housed (*left*; $n = 10$) and individually housed (*right*; $n = 11$) males that showed increases in serotonergic activity during social interaction. Vertical gray lines depict the 50% rise times for each individual. Although more socially housed males had low rise times, the difference in rise times between groups is not significant due to the wide variation in socially housed males (1-way ANOVA, $F_{1,20} = 1.017$, $P = 0.326$).

Social housing influenced serotonin-behavior relationships. Because past work has shown that serotonergic activity is related to social and nonsocial behaviors in male mice encountering an intruder (Hall et al. 2011), we examined whether serotonin-behavior relationships were affected by social experience. For group-housed males, mean serotonergic activity was negatively correlated with inactivity (Spearman correlation, $r_s = -0.698$, $n = 13$, $P = 0.008$; Fig. 4*A*), a measure that was defined as subject males being completely immobile, as well as having no contact with the intruder male. In other words, males that were more inactive exhibited a smaller increase or even a decrease in serotonergic activity. In contrast to this relationship, individually housed males did not exhibit a correlation between mean serotonergic activity and inactivity (Spearman correlation, $r_s = 0.029$, $n = 14$, $P = 0.923$; Fig. 4*B*). Furthermore, the correlation coefficients for the relationships between serotonergic activity and resident inactivity were significantly different between individually and group-housed males (Fisher's *r*-to-*z* test, $z = -2.042$, $P = 0.041$).

Mean serotonergic activity was positively correlated with nose-to-nose investigation for group-housed males ($r_s = 0.709$, $n = 13$, $P = 0.007$; Fig. 4*C*). In contrast, the correlation between mean serotonergic activity and nose-to-nose investigation did not reach significance in individually housed mice ($r_s = 0.477$, $n = 14$, $P = 0.085$; Fig. 4*D*), and this correlation was also not significant after the outlier was removed ($r_s = 0.451$, $n = 13$, $P = 0.122$). However, the serotonin-investigation correlation coefficients for group-housed and individually housed males were not statistically different (Fisher's *r*-to-*z* test, $z = 0.838$, $P = 0.402$).

There were no significant correlations for either group-housed and individually housed males between mean serotonergic activity and the following behaviors: resident anogenital investigation, intruder anogenital investigation, mounting by the intruder, bucking, total USVs, proportional use of 50-kHz USVs, and total number of broadband squeaks. The correlations between serotonergic activity and the resident subject animal's weight for group-housed animals ($r_s = 0.545$, $n = 13$, $P = 0.054$) or individually housed animals ($r_s = 0.095$, $n = 14$, $P = 0.095$) did not reach significance.

Because the two housing treatments differed in timing but not amplitude, we further explored the relationship between a measure of the serotonergic trajectory, 50% rise time, and a measure of vocal behavior, the peak percentage of 50-kHz

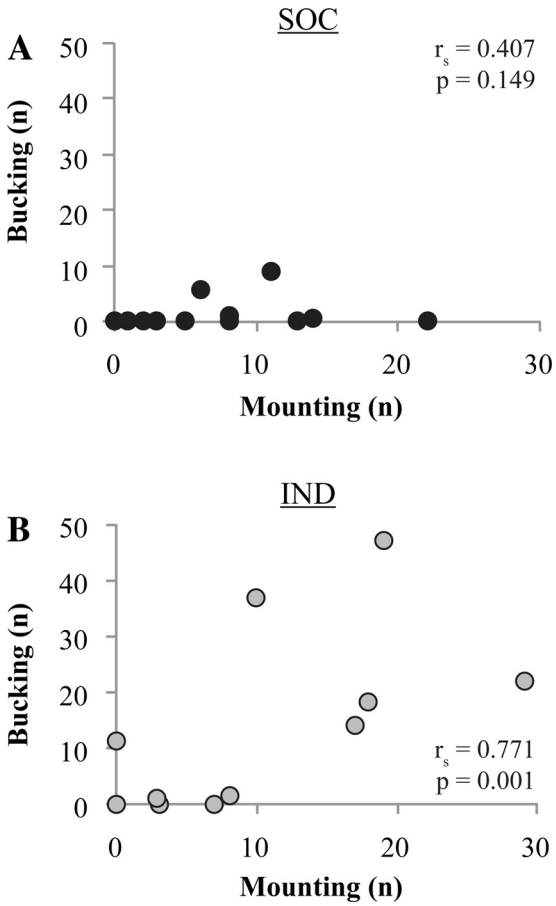

Fig. 2. *A*: correlation between mounting performed by male intruder mice and bucking exhibited by resident male subjects previously housed in social groups (n = 9 mice). *B*: correlation between mounting performed by male intruder mice and bucking exhibited by resident male subjects previously housed individually (n = 10 mice). r_s, Spearman rank correlation coefficient.

USVs normalized to all USVs. The 50-kHz USVs are behaviorally potent, corresponding to mounting behavior in male mice interacting with either males or females (Finton et al. 2017; Hanson and Hurley 2012, 2014; Keesom et al. 2017). Figure 5 illustrates the relationship between 50% rise times and the peak proportion of 50-kHz USVs. The correlation between these two variables did not reach significance (Spearman correlation, $r_s = -0.397$, $n = 21$, $P = 0.075$).

DISCUSSION

Social experience plays an important part in shaping the structure and function of neurochemical systems in the brain (Cacioppo et al. 2015; Fone and Porkess 2008; Lukkes et al. 2009). In this study, we tested whether social housing conditions influence socially induced serotonergic activity in the IC (auditory midbrain). Our findings did not support our initial prediction that group-housed males would exhibit a greater elevation in serotonergic activity than individually housed males. Instead, although males from both housing treatments demonstrated elevated serotonergic activity during social intrusions, we found that social housing affected the timing of the serotonergic trajectory. Timing was affected in two ways: *1*) group-housed male mice exhibited elevated serotonergic activity 12 min before individually housed males, and *2*) group-housed male mice exhibited maximal serotonergic activity by ~10 min sooner than individually housed males. We also found that housing conditions altered relationships between serotonergic activity and behavior. For group-housed males, serotonergic activity and nose-to-nose investigation were positively correlated, and serotonergic activity and behavioral inactivity were negatively correlated. In contrast, individually housed males did not demonstrate relationships between serotonergic activity and any of the behaviors we measured. These findings suggest that the social experience gained through social housing may play an important role in establishing the central serotonergic system as a signal of social context.

Social isolation delayed elevations in socially induced serotonergic activity. Similar to our finding, the influence of social isolation on serotonin release has been demonstrated across the brain, with the direction of effect depending on the region of interest and the stimulus. Relative to group housing, individual housing attenuates the release of serotonin triggered by nonsocial stressors in the hippocampus and frontal cortex (Bickerdike et al. 1993; Muchimapura et al. 2002). In contrast, individual housing results in elevated release of serotonin triggered by a conspecific intruder in the prefrontal cortex (Ago et al. 2013) and by a mild foot shock in the nucleus accumbens (Fulford and Marsden, 1998). Our finding of a change in the timing of serotonergic activity also has a parallel in the responses of male anoles to the acute experience of winning or losing an agonistic interaction with another male (Summers 2002; Summers et al. 2003; Ling et al. 2009). Individuals that experience a win (dominant males) vs. a loss (subordinate males) exhibit different temporal patterns in central monoaminergic activity, in that losers exhibit delayed physiological responses to social intrusions, as well as nonsocial stressors (Ling et al. 2009; Summers 2002; Summers et al. 2003). For example, losers exhibit a slower elevation in serotonin turnover [5-hydroxyindoleacetic acid (5-HIAA)/5-HT] in the raphé nuclei in response to a social intrusion, although losers achieve the same level as winners after 40 min; this pattern is similar for DOPAC, a metabolite of dopamine, in the raphé nuclei as well (Summers et al. 2003). In the medial amygdala, an area important for the expression of aggressive behavior, dominant and subordinate animals differ in the timing of serotonergic elevations on a scale from 10 min to 1 wk (Summers et al. 2003). In other vertebrate groups, serotonin may also correspond to social status. In talapoin monkeys (*Miopithecus falapoin*), for example, the levels of a serotonergic metabolite in the cerebrospinal fluid increase as individuals become subordinate but decrease as individuals become dominant. Furthermore, in subordinates, the serotonergic metabolite is not responsive to aggression (Yodyingyuad et al. 1985). We did not specifically find an effect of social status on serotonergic activity, but these findings generally indicate that past social experience acquired by the outcome of a fight or through group vs. individual housing alters the timing of physiological responses to stressors, such as being placed with a social intruder.

Mechanistically, social experience could have altered the release of serotonin by acting at multiple levels (Fig. 6). The IC receives most of its serotonergic fibers from the dorsal raphé nucleus (Klepper and Herbert 1991). At the level of this

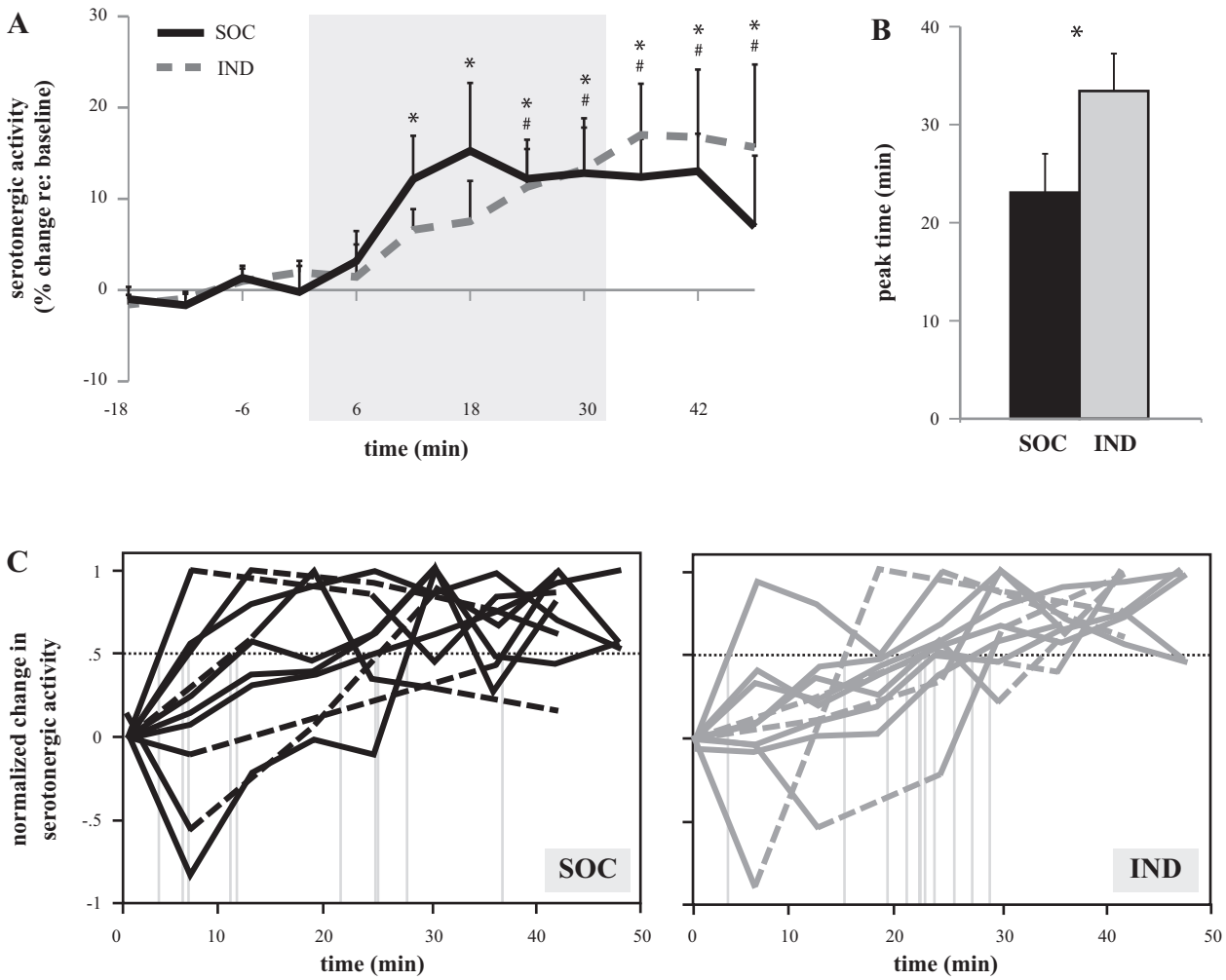

Fig. 3. *A*: time courses of serotonergic activity in the IC of resident male mice from different social histories (SOC, housing in social groups, $n = 9$; IND, individual housing, $n = 10$). Mean serotonergic activity significantly increased in male mice when paired with a male intruder, compared with baseline values (linear mixed model, $P < 0.01$) (pairwise comparisons to baseline: IND, $*P < 0.05$; SOC, $\#P < 0.05$). Gray box indicates presentation of the male intruder (0–30 min). Values are means ± SE. *B*: the time of maximal serotonergic activity (peak time) after presentation of the intruder was significantly earlier for resident males previously housed in social groups (SOC; $n = 9$) compared with resident males previously housed individually (IND; $n = 10$). Bars represent means ± SE (2-way ANOVA, $*P < 0.05$). *C*: normalized trajectories of serotonergic activity over time for individual male mice. Dashed lines are interpolated trajectories at time points when single measurements were not possible because of excessive movement noise. Vertical gray lines mark 50% rise times for each male. *Left*, socially housed animals (black; $n = 10$ experiments); *right*, individually housed animals (gray; $n = 11$ experiments).

nucleus, there is functional variation in subpopulations of raphé neurons with different terminal fields and inputs, including inputs from regions stereotypically involved in social processing (Crawford et al. 2013; Hale et al. 2012; Kirifides et al. 2001; Lee et al. 2007; Pollak Dorocic et al. 2014; Vertes and Linley 2008). An effect of social experience on the regulation of neural excitability by social stimuli at the level of the dorsal raphé nucleus or upstream is therefore a plausible mechanism for the effects we observed. Although most of the serotonergic innervation of the IC originates in the dorsal raphé nucleus (Klepper and Herbert 1991), the organization of this input among functionally distinct raphé subpopulations is not known. Collaterals from single raphé neurons are capable of making widespread contact in functionally related brain regions, however (Lee et al. 2008), so it is possible that serotonin release in the IC is coupled to serotonergic events at other sites. Indeed, patterns of serotonergic activity in regions such as the preoptic area show striking similarities to our measurements in the IC in some contexts. In male rats, voltammetrically measured serotonin in the preoptic area increases during the consummatory phase, but only when female social partners are sexually available (Fumero et al. 1994; Mas et al. 1995), similar to our findings in the IC of male mice placed with female partners (Keesom and Hurley 2016).

Local mechanisms are also capable of regulating serotonin at the extracellular level. For example, the 5-HT_{1B} receptor sometimes acts as a terminal autoreceptor inhibiting serotonin release (Sari 2004). Likewise, the serotonin transporter is relatively selectively expressed by serotonergic fibers themselves, and strongly influences serotonin levels and the time course of serotonin availability (Fujita et al. 1993; Hashemi et al. 2012; Nielsen et al. 2006). An effect of social isolation on the expression or function of these local regulatory molecules could therefore also have resulted in at least some of the changes we observed.

Social isolation alters relationships between serotonergic activity and behavior. The finding that the physiological variable of serotonin corresponds to social behavior in group-

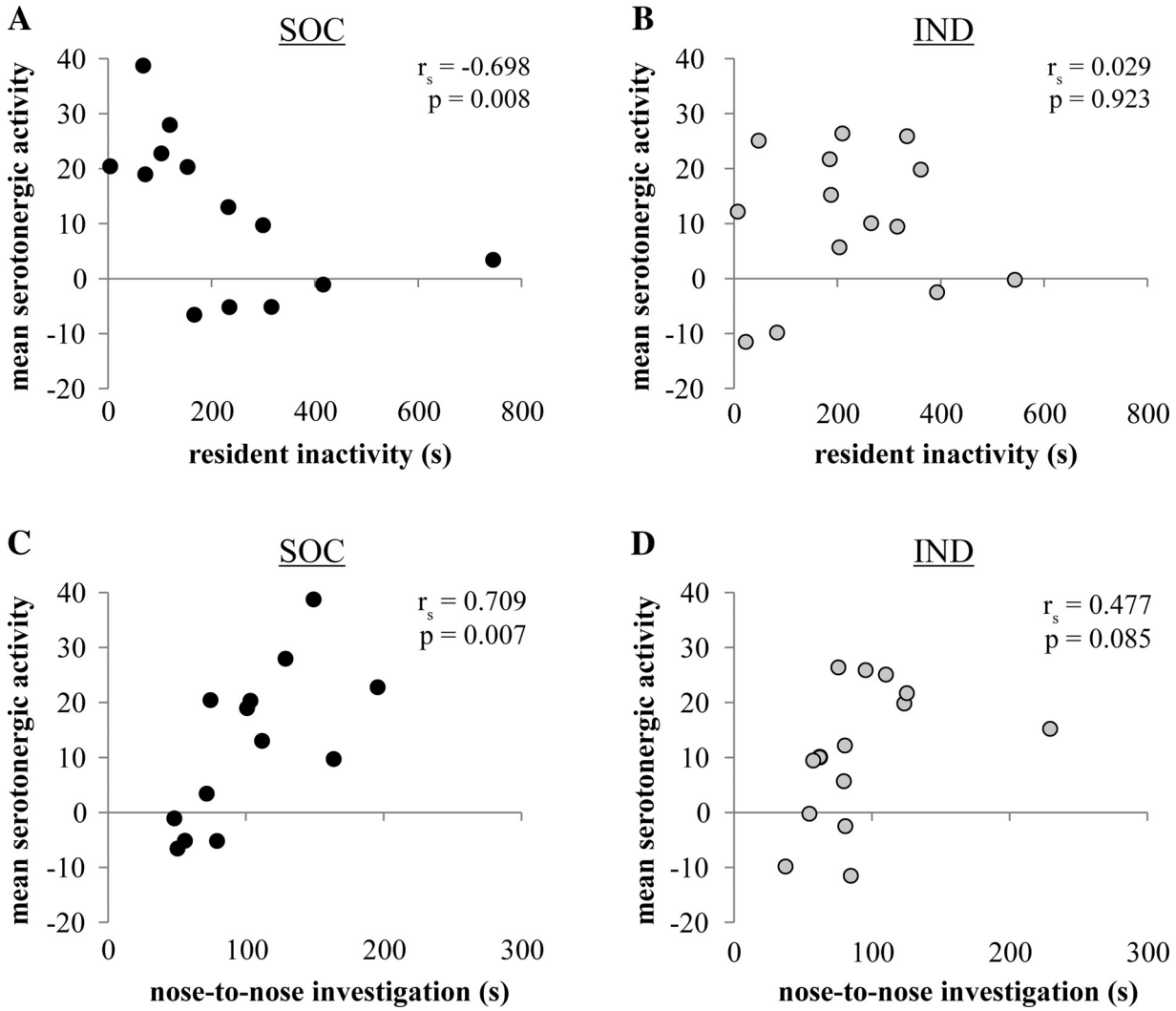

Fig. 4. *A* and *B*: correlations between mean serotonergic activity and resident inactivity for males housed in social groups (SOC; $n = 9$ mice; *A*) and males housed individually (IND; $n = 10$ mice; *B*). *C* and *D*: correlations between mean serotonergic activity and nose-to-nose investigation for males housed in social groups (SOC; $n = 9$ mice; *C*) and males housed individually (IND; $n = 10$ mice; *D*). r_s, Spearman rank correlation coefficient.

housed, but not individually housed, mice is closely related to the idea of social competence: that behavior grades to match a particular social situation. A fine-tuned response may permit animals to use more "costly" behaviors only when it is warranted by the current situation (Taff and Vitousek 2016). This could include the use of aggressive signals instead of attacking and engaging in a fight with a conspecific animal, which could be costly in terms of both energy expenditure and physical injury. In one example, social experience gained via group housing in male convict cichlids (*Archocentrus nigrofasciatus*) permits a correspondence between postfight plasma cortisol and the intensity of the encounter during agonistic interactions, although this relationship was found only for "losers" (Earley et al. 2006). Individually housed "winners" and individually housed "losers," as well as group-housed "winners," did not exhibit this relationship between plasma cortisol and encounter intensity. This finding suggests that a graded physiological response, associated with a graded behavioral response, depends on experience with conspecifics.

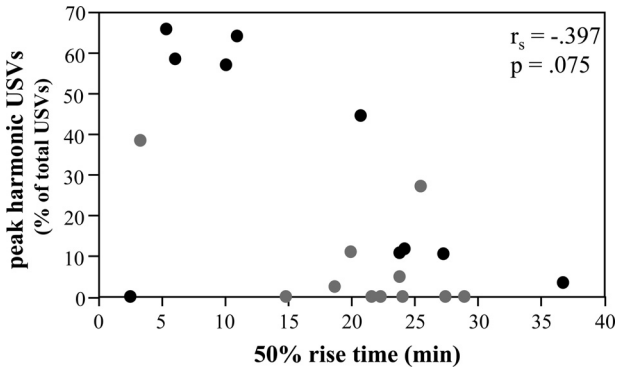

Fig. 5. Serotonergic trajectory corresponds to behavior. Plotted are 50% rise times vs. the peak percentage of 50-kHz USVs in a single 6-min time bin for each individual. Black circles represent socially housed animals; gray circles represent individually housed animals.

Our results illustrate that sensory systems are integrated into this process. In the past, we have found that serotonergic elevations in the IC during same-sex social encounters between males are positively related to social behavior, as well as negatively related to inactivity, of the subject resident male (Hall et al. 2011). In contrast, in male mice placed with female social partners, serotonergic activity of male subjects is nega-

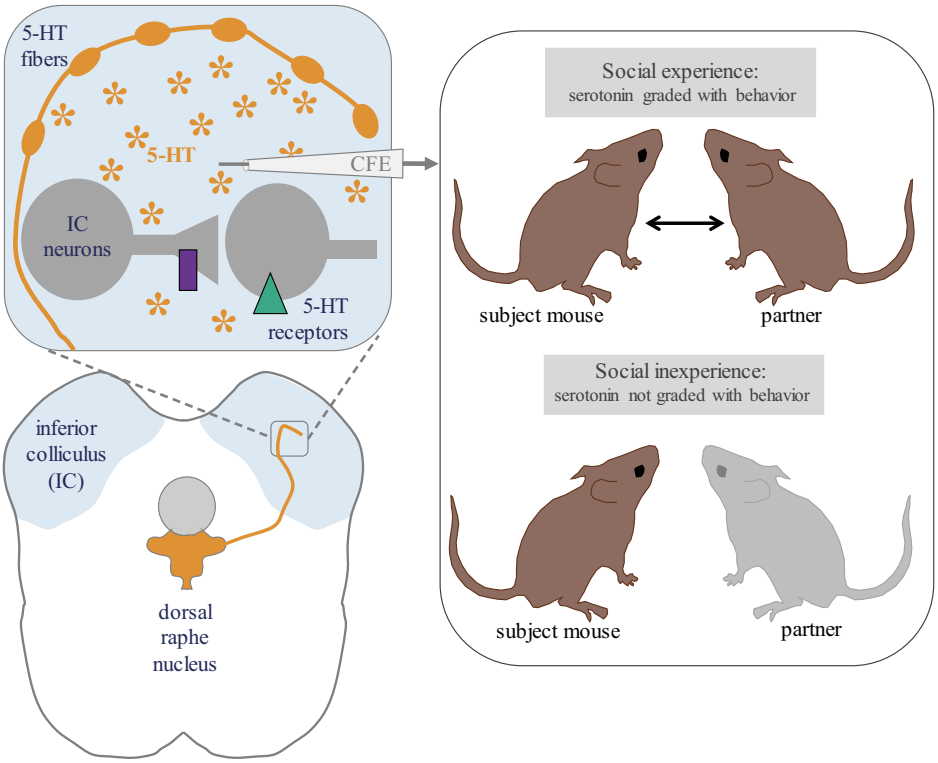

Fig. 6. Effects of social isolation on serotonergic activity in the IC measured through carbon fiber electrodes (CFEs) could occur upstream of the IC or through local mechanisms. Schematic representation shows the relative locations of the IC and dorsal raphé nucleus in the transverse plane (*bottom left*) and of serotonin release in the IC (*top left*). Social experience results in serotonin release correlating with behavioral interactions with a partner (*right*).

tively related to signals of rejection by the female social partner, but not to male subject behavior (Keesom and Hurley 2016). In the present study, we found that group-housed male mice interacting with a novel male intruder exhibited a positive relationship between serotonergic activity and social investigation (nose-to-nose investigation), as well as a negative relationship between serotonergic activity and inactivity, thus corroborating the findings of Hall et al. (2011). In contrast, individually housed mice in the current study did not exhibit relationships between serotonergic activity and any of the behaviors we measured. This was not simply due to differences in the levels of social motivation by individually housed and group-housed males, because there was no difference in social investigation between these treatments. The absence of serotonin-behavior relationships could also be due to altered variability in the serotonergic response, where individually housed males exhibit socially induced serotonergic activity as an all-or-nothing response. However, the individually housed males in the current study exhibited variability in serotonergic activity that was not distinct from variability exhibited by group-housed males. These findings suggest that social experience gained through group housing may contribute to the establishment of relationships between serotonergic activity and behaviors.

Cross-disciplinary models that overlap with the concept of social competence have been developed to account for variable relationships between physiology and behavior from multiple disciplinary perspectives. One of these models views short-term adjustments in the physiological variables underlying behavior as "rapid endocrine flexibility," a trait subject to natural selection that can therefore result in adaptive behavior (Taff and Vitousek 2016). Key features of this model are an emphasis on within-individual differences in response to internal or external events and behaviorally meaningful changes in both the amplitude and timing of physiological variables. A conceptual comparison that combines both chronic and acute effects of the social environment is found in the phenomenon of "social buffering," in which social housing can ameliorate responses to stressful events (Fox et al. 2006; Reinelt et al. 2014). Although the roles of multiple neurochemical systems in social buffering have been explored (Lieberwirth and Wang 2016; Tabbaa et al. 2016), the serotonergic system may be sensitive to the conditions that create social buffering in behavior. In the mouse prefrontal cortex, repeated social defeat increases the level of 5-HIAA, a major metabolite of serotonin, in mice that have been housed individually relative to group-housed mice, suggestive of a buffering effect of social housing (McQuaid et al. 2013). Within the individually housed group, an enriched physical environment reduces the effects of social defeat so that the influence of social and physical environments interact (McQuaid et al. 2013). Both of these models validate the idea that the effects of social experience on serotonergic activity in our system may play a role in the response to acute social interaction.

Potential consequences of altered timing and serotonin-behavior relationships for social competence. The delayed elevation in socially induced serotonergic activity of individually housed mice, as well as the absence of serotonin-behavior relationships, could have potentially critical consequences for the perception of the auditory signals used during these interactions. In this study, male mice emitted both ultrasonic vocalizations and broadband squeaks. During male-male agonistic encounters, squeaks are a particularly important signal, because they are emitted under both asocial and social states of duress (Gourbal et al. 2004; Whitney and Nyby 1983) and contain structural variations that could relay additional information regarding the signaler's motivational/arousal state (Fitch et al. 2002; Plekhanova and Egorova 2013). Relatively

little is known about the influence of serotonin on auditory processing at the perceptual/behavioral level; however, studies demonstrate serotonin's action as an auditory neuromodulator at lower organizational levels. At the level of single cells, locally applied serotonin influences the selectivity of auditory neurons for social vocalizations, tending to make them more selective (Hurley and Pollak 2005). Furthermore, exogenous administration of serotonin modulates the activity of the auditory system at a population level (Papesh and Hurley 2016). One study in weakly electric fish (*Apteronotus leptorhynchus*) suggests that serotonin is a strong modulator of the perception of social signals used during aggressive encounters (Deemyad et al. 2013). Deemyad et al. found that both endogenously released and locally applied serotonin enhanced the activity of electrosensory neurons to electric signals mimicking signals used during agonistic encounters, as well as the behavioral response to these signals (Deemyad et al. 2013). Thus elevations in serotonergic activity that depend on current context may facilitate fine-tuning of auditory processing to the context, with the potential consequence of matching behavioral output to the particular interaction (Fig. 6).

Summary. In this study, we have shown that social experience influences how serotonergic activity reflects external social context in an auditory region. The delayed elevation in serotonergic activity and absence of serotonin-behavior relationships exhibited by individually housed males demonstrate that experience plays an important role in shaping context-dependent physiological responses. Thus the centralized serotonergic system is one mechanism that can integrate past experience with current behavioral context.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.M.K. and L.M.H. conceived and designed research; S.M.K. and Z.E.-B. performed experiments; S.M.K., B.G.S., Z.E.-B., and L.M.H. analyzed data; S.M.K. and L.M.H. interpreted results of experiments; S.M.K. and L.M.H. prepared figures; S.M.K. drafted manuscript; S.M.K., B.G.S., Z.E.-B., and L.M.H. edited and revised manuscript; S.M.K., B.G.S., Z.E.-B., and L.M.H. approved final version of manuscript.

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