BRIEF COMMUNICATIONS

Sexual Orientation-Related Differences in Prepulse Inhibition of the Human Startle Response

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Prepulse inhibition (PPI) refers to a reduction in the startle response to a strong sensory stimulus when this stimulus is preceded by a weaker stimulus—the prepulse. PPI reflects a nonlearned sensorimotor gating mechanism and also shows a robust gender difference, with women exhibiting lower PPI than men. The present study examined the eyeblink startle responses to acoustic stimuli of 59 healthy heterosexual and homosexual men and women. Homosexual women showed significantly masculinized PPI compared with heterosexual women, whereas no difference was observed in PPI between homosexual and heterosexual men. These data provide the first evidence for within-gender differences in basic sensorimotor gating mechanisms and implicate the known neural substrates of PPI in human sexual orientation.

The past decade has seen an explosion of high-profile findings concerning the biological correlates of human sexual orientation. Although there is no single etiogenic account of human sexual orientation, often such findings have arisen within the context of the theory of prenatal hormonal sexual differentiation, which proposes that the development of neurobehavioral differences between the two sexes is under the control of sex steroids. Homosexuals are considered to follow sex-atypical patterns (in the direction of the opposite sex) of development in neurobehavioral domains as evidenced by the “atypical” shift in their preference for sexual partner (Ellis & Ames, 1987). Findings supportive of this notion include observations that homosexual men have larger neuronal populations in the suprachiasmatic nucleus (Swaab & Hofman, 1990), smaller interstitial nuclei of the anterior hypothalamus (LeVay, 1991, cf. Byne et al., 2001), larger anterior commissures (Allen & Gorski, 1992), larger isthmus of the corpus callosum (Scamvougeras et al., 1994), and female-typical performance on cognitive tests that typically differentiate men and women, such as mental rotation and verbal fluency abilities (C. M. McCormick & Witelson, 1991; Rahman & Wilson, 2003a; Wegesin, 1998b). In neurophysiological studies, homosexual men show greater inhibition of alpha activity (recorded from electroencephalography) across the right hemisphere during a verbal task (Alexander & Sufka, 1993), symmetrical auditory source locations in the superior temporal gyrus (measured by magnetoencephalography, Reite, Scheeder, Richardson, & Teale, 1995), and female-typical slow-wave activity during performance on mental rotation (using event-related potentials, Wegesin, 1998a) compared with heterosexual men. However, homosexual men also show what could be considered sex-atypical neurobehavioral responses in the opposing direction. For example, McFadden and Champlin (2000) reported that the auditory brainstem response (ABR) components of auditory evoked potentials (AEPs), indicative of brainstem activity, were hyper-masculinized in homosexual men compared with heterosexual men. Similarly, in homosexual women, what evidence there is for a cross-sex shift is inconsistent, although studies of homosexual women are few in number. Investigations of cognitive performance show no differences between homosexual and heterosexual women in spatial and verbal performance, but there is evidence of masculinized targeting performance in the former group (Hall & Kimura, 1995). Homosexual women also show a trend toward high levels of slow-wave activity during mental rotation tasks, similar to heterosexual men (Wegesin, 1998a), and show masculinized AEPS (McFadden & Champlin, 2000). This pattern of results is suggestive of underlying neural variation.

Several lines of evidence suggest that human homosexuality (and by inference its neurobehavioral correlates) may, in part, be influenced by prenatal factors, particularly the levels of androgens experienced by the individual in utero. Homosexual women exhibit a smaller second-to-fourth finger length ratio (a masculinization; McFadden & Shubel, 2002; Rahman & Wilson, 2003b; Williams et al., 2000), and homosexual men also have been reported to exhibit a smaller second-to-fourth finger length ratio than heterosexual men (a hypermasculinization; see Rahman & Wilson, 2003b; Robinson & Manning, 2000, but cf. Williams et al., 2000; McFadden & Shubel, 2002). Further, homosexual women and men both also exhibit a higher incidence of left-handedness (Lalumiere, Blanchard, & Zucker, 2000). There is also evidence for modest heritability of male and female homosexuality (Bailey, Dunne, &
Martin, 2000) and linkage to markers in the Xq28 chromosomal regions in men, but not in women (Hu et al., 1995, cf. Rice et al., 1999). There is also evidence that the auditory response mechanisms of lesbians and gay men may be influenced by prenatal factors. Homosexual women have consistently been shown to have masculinized (i.e., less numerous and weaker) otoacoustic emissions (OAEs: weak sounds produced by the inner ear) compared with heterosexual women, although no differences are found between homosexual and heterosexual men (McFadden & Pasanen, 1998, 1999). A role for early androgens in these differences is suggested by the finding that females with male co-twins (and therefore perhaps more exposure to androgens) have OAEs more like males than those of other females (McFadden, 1993). If the findings with regard to finger length ratios and OAEs are considered with the differences in AEPs, then they imply, rather counterintuitively, that androgenic influences have localized effects on male sexual orientation. On the other hand, the pathway for women appears linear: elevated prenatal androgens leading to masculinized neurobehavioral (including direction of sexual preference, i.e., choosing women as mates) profiles (McFadden, 2002; Rahman & Wilson, 2003b).

Although prenatal factors may be possible precursors to the neurobehavioral profiles observed in lesbians and gay men, whether neural differences underlie sexual orientation per se, or are a consequence of homosexual or heterosexual behavior, is yet to be determined. The extent to which these are due to “hard-wiring” or the influence of learning on the developing brain is yet to be elucidated.

One neurobehavioral domain that may be informative on this question involves sexually dimorphic startle responses. Modulation of the startle response represents a cross-species, noninvasive psychophysiological task that can be easily applied to evaluate individual differences in attentional–information processing. Pre-pulse inhibition (PPI) refers to a reliable reduction in the startle response to a strong sensory stimulus (pulse) when it is preceded at an interval of 30–500 ms by a weak stimulus—the prepulse (Graham, 1975). This phenomenon is thought to reflect a sensorimotor gating mechanism, protecting the organism from behavioral interference that would otherwise result from simultaneous processing of discrete stimuli.

In rat models, PPI has an established neural basis in limbic and cortico–pallido–striato–thalamic circuitry (Swerdlow, Geyer, & Braff, 2001). Specifically, there is evidence for the involvement of the hippocampus, nucleus accumbens, striatum, amygdala, ventral pallidum, globus pallidus, substantia nigra, thalamus, pedunculopontine nucleus, and the superior and inferior colliculus (Swerdlow et al., 2001). Consistent with the known neural substrates of PPI in the rat, deficient PPI is observed in a number of psychiatric and neurological disorders in humans characterized by abnormalities in cortico–pallido–striato–thalamic circuitry, including schizophrenia (Braff, Swerdlow, & Geyer, 1999), obsessive–compulsive disorder (Swerdlow, Benbow, Zisook, Geyer, & Braff, 1993b), and Tourette’s syndrome (Castellanos et al., 1996). In particular, the striatum is a key region responsible for inhibitory processing, whereas the hippocampus and thalamus are centrally implicated in the control of sensory gating in humans (Castellanos et al., 1996; D. A. McCormick & Bal, 1994). In healthy humans, neuroimaging studies have shown involvement of subcortical and cortical regions involving primarily the basal ganglia, thalamus, hippocampus, the prefrontal and parietal cortex in PPI (Kumari et al., in press). Robust sex differences have also been observed in humans and rats, with females exhibiting lower PPI than males in both species (Faraday, O’Donoghue, & Grunberg, 1999; Swerdlow, Auerbach, et al., 1993).

PPI is nonlearned and is observed early in humans and in animals, including invertebrates (Hoffman & Ison, 1992). PPI is known to occur on the very first exposure to pulse-alone and prepulse stimuli, indicating that it is not a form of conditioning or learning (Swerdlow et al., 1992, 1994). Although the eyelid response to startle probes shows habituation over repeated presentation of startle probes in normal subjects, it can still be reliably elicited and measured (with the methods and stimulus parameters used in this study) in a study sessions of over 100 trials (Braff et al., 1978). The PPI effect is known to show high stability (within and between sessions) in healthy individuals (Cadenhead, Carasso, Swerdlow, Geyer, & Braff, 1999). Studies in rats have also indicated the stability of PPI with repeated pulse-alone and prepulse + pulse trials with stimulus parameters such as those used here (Gewirtz & Davis, 1995).

Given the evidence for cross-sex shifts shown by lesbians and gay men in certain neurobehavioral functions, the apparent role of prenatal androgens for organizational effects on human sexual orientation, and the evidence that components of sensorimotor function are also influenced by sex steroids, the current study aimed to examine whether PPI might also differ between heterosexuals and homosexuals. Because PPI has established neural correlates in humans and nonhuman animals, exploring possible sexual orientation–related differences in PPI provides a unique opportunity for furthering our understanding of the neural circuitry underlying sexual orientation and its developmental pathways.

Method

Participants

Participants were 59 healthy heterosexual and homosexual men and women (on whom data are reported). Heterosexual participants were recruited from university sources, through newspaper advertisements, and social networks, and homosexual participants were recruited through student lesbian and gay societies, newspaper advertisements in lesbian and gay publications, and social networks. Thus, all groups of participants were recruited from similar sources. All participants came from within the London and Southeast regions of England. Participants were screened to ensure no psychiatric or neurological illness or drug use. This was done by asking participants a general screening question on psychiatric and neurological illness, with examples provided (depression, panic attacks, schizophrenia, epilepsy, and head injuries or concussion). Any participants stating that they had a history, or any indication, of psychiatric or neurological illnesses were not recruited into the study. Sexual orientation was assessed with a single-item, Kinsey-style sexual orientation measure (Coleman, 1987). All included participants were screened for normal hearing at 1000 Hz by means of an audiometer (Kampllex, AS7; PC Werth Ltd., London, UK) set to 40 dB(A) prior to being accepted as study participants. One participant was excluded from analysis. Startle response data were discarded for 3 heterosexual men, 3 heterosexual women, 3 homosexual men, and 1 homosexual woman because of poor quality (response probability over pulse-alone trials < 70%). This left 15 heterosexual men (mean age = 25.40, SD = 3.48), 15 homosexual men (mean age = 31.66, SD = 6.10), 15 heterosexual women (mean age = 24.73, SD = 3.21), and 14 homosexual women (mean age = 29.07, SD = 5.83) selected out.
of an original sample of 70. The groups differed significantly in age, with homosexual men being older than the other groups, F(3, 58) = 6.79, p < .01.

**Startle Response Measurement**

A commercial human startle response monitoring system (Mark IL, Startle Reflex Lab; San Diego Instruments, San Diego, CA) was used to generate and deliver the startle stimuli and to record and score the electromyographic (EMG) activity for 250 ms starting from the onset of acoustic startle stimuli. Acoustic stimuli were presented binaurally through headphones (Telephonics, TDH – 39P), EMG recordings were taken with participants sitting comfortably in a dimly lit laboratory. The experimental procedures and scoring of the startle reflexes were identical to those used in previous studies (Kumari, Soni, Mathew, & Sharma, 2000). The startle reflex component of the startle response was recorded via the EMG activity of the orbicularis oculi muscle directly beneath the right eye with two silver/silver chloride (Ag/AgCl) 6-mm electrodes filled with Dracard electrolyte paste (SLE, Croydon, UK). A third electrode was the ground reference electrode placed behind the right ear, over the mastoid.

Following a 5-min acclimatization period during which a 70-dB white noise was presented, stimuli were presented as a pulse-alone, 40-ms presentation of an 115-dB white noise and a prepulse of 20-ms presentation of an 84-dB white noise, over a 70-dB continuous background noise. Participants received 32 stimuli. The first 4 and the last 4 were pulse-alone trials. The remaining were divided into 4 blocks of 6 trials each. Each block consisting of 3 pulse-alone and 3 single prepulse (pulse onset to prepulse onset interval = 120 ms) trials. The mean intertrial interval was 15 s (range = 9–23 s), as in numerous PPI studies (with longer sessions and many more trials), in both normal and clinical populations, that used exactly the same parameters and measurements methods (e.g. Braff et al., 1978, Braff, Grillion & Geyer, 1992; Grillion, Ameli, Charney, Krystal, & Braff, 1992; Kumari, Checkley, & Gray, 1996, Kumari, Soni, & Sharma, 1999, Kumari et al., 2000, 2001).

EMG activity was bandpass filtered to eliminate 50-Hz interference. Raw data were visually inspected for each trial for every subject and only scored if there was clear evidence of an eyeblink within 150 ms of the onset of the pulse-alone stimulus. Fewer than 5% of the responses were rejected because of no clear evidence of an eyeblink. The EMG data on valid trials (i.e., with clear eye blinks) were then scored offline by the (automatic) analytic program of the Startle Reflex lab (by author Veena Kumari, who was blind to participants’ sex and sexual orientation group membership) for response amplitude (maximal amplitude that occurred within 150 ms after the acoustic stimulus, in arbitrary analog-to-digital units; 1 unit = 2.62 μV). Raw data for peak amplitude (in arbitrary analog-to-digital units) over six pulse-alone and six prepulse-plus-pulse trials for each subject were averaged (separately for pulse-alone and prepulse-plus-pulse conditions) and used to calculate the PPI as pulse-alone amplitude minus amplitude over prepulse-plus-pulse trials divided by pulse-alone amplitude.

**Data Analysis**

For analysis of group differences in percent PPI, the data were subjected to a general linear model (GLM) one-way analysis of variance (ANOVA), with group (Sex × Sexual Orientation Status) as the between-subjects variable. Significant effects were followed up by post hoc comparisons with Fisher’s least significant difference test. For analysis of amplitude and habituation, the 115-dB pulse-alone amplitude data over the four blocks were subjected to a two-way Group × Block GLM ANOVA. Absence of group effect (see Results) in response habituation was further confirmed through the analysis of percent reduction in mean amplitude from the first to the last block of pulse-alone trials with a one-way GLM ANOVA. Effect sizes (Cohen’s d; mean for one group minus the mean for the other group divided by the averaged standard deviation)—where 0.2 is a small effect, 0.5 a medium effect, and 0.8 a large effect (Cohen, 1988)—are also presented. All statistical analyses were performed with SPSS (Version 10; SPSS, Chicago, IL). Alpha level for significance testing was 5%.

**Procedure**

Participants were informed that the study aimed to investigate gender differences in reactivity to loud noises and information processing. The experimental procedures (electrodes and site of positioning) were explained, and participants were told that they were going to hear a number of auditory clicks over approximately 30 min. They were requested to relax, but to stay awake. Participants were instructed neither to ignore nor to attend to the noise bursts heard over the headphones. All participants gave their written informed consent to take part after the aim and procedure of the study had been explained to them. They were debriefed at the end of the testing session. The Ethical (Research) Committee of the Institute of Psychiatry and Maudsley Hospital, London approved all procedures with human participants.

**Results**

Table 1 shows the mean eyeblink startle amplitudes for the four blocks (six trials) of pulse-alone trials and four blocks (six trials) of prepulse-plus-pulse (i.e., where prepulse preceded the pulse by 120 ms) trials. Figure 1 shows the mean percentage of PPI across the groups. There were no significant group differences in response amplitude, as indicated by the lack of significant group effect, F(3, 55) = 0.53, p = .66, or habituation over the block of pulse-alone trials, as shown by a lack of significant Group x Block effect, F(3, 165) = 1.45, p = .17. Conferring the lack of group effect in response habituation, groups again did not differ in terms of percent reduction in mean response amplitude from the first block to the last block of trials, F(1, 55) = 1.51, p = .22.

There were significant group differences in percentage of PPI, F(3, 55) = 3.11, p = .03, demonstrating (a) masculinized inhibition in homosexual women (who were significantly different from heterosexual women, p = .03, d = 0.83), and (b) the established lower inhibition in heterosexual women compared with heterosexual men (p < .01, d = 1.11). Homosexual men did not differ significantly from heterosexual men (p = .51), although the size of this nonsignificant difference (in a female-typical direction) was a small effect (d = 0.23).

**Discussion**

Our results show, for the first time, that PPI relates to sexual orientation and that homosexual women show a robust cross-sex shift. Homosexual women showed a masculinized PPI that was no different from that of heterosexual men. These differences were large by a standard criterion (Cohen’s d). Homosexual men did not differ from heterosexual men. No differences were seen in response habituation or response amplitude, indicating that the observed PPI differences were not due to related effects in other startle characteristics.

The present findings confirm the established sex effects demonstrated in PPI in humans and rats (Faraday et al., 1999; Swerdlow, Auerbach, et al., 1993), with females showing less PPI than males. Further, our results show for the first time that this normative sex difference is specific to heterosexual men and women. Moreover, the masculinization of PPI shown by homosexual
Table 1

Mean (±SD) Eyeblink Startle Amplitudes for Blocks of Pulse-Alone and Prepulse-Plus-Pulse Trials Across Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Amplitude for 4 blocks of pulse-alone trials</th>
<th>Amplitude for 4 blocks of prepulse-plus-pulse trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>336.80 ± 231.86</td>
<td>318.42 ± 258.40</td>
</tr>
<tr>
<td>Heterosexual women</td>
<td>355.35 ± 267.71</td>
<td>310.04 ± 264.26</td>
</tr>
<tr>
<td>Homosexual men</td>
<td>431.95 ± 269.45</td>
<td>351.75 ± 259.52</td>
</tr>
<tr>
<td>Homosexual women</td>
<td>489.95 ± 360.32</td>
<td>359.80 ± 210.37</td>
</tr>
</tbody>
</table>

Note. In prepulse-plus-pulse trials, the prepulse preceded the pulse by 120 ms. P = pulse alone; PP = prepulse plus pulse.
However, possible neurodevelopmental explanations for the present results are not limited to those implicating purely hormonal effects. It is possible that sexual orientation-related differences in PPI could be due to prenatal stress; the actions of environmental agents such as nicotine or alcohol, or those that mimic the actions of sex hormones; or to developmental instability. Developmental instability (the extent of perturbation in morphological symmetry experienced by an organism) is an unlikely explanation, as studies show no differences in fluctuating asymmetry (a measure of developmental instability) between heterosexuals and homosexuals (Rahman & Wilson, 2003b). However, there is some evidence for differences in maternal reports of prenatal stress experienced during the 1st and 2nd months of pregnancy between homosexuals and heterosexuals, and the mothers of homosexual women report higher consumption of nicotine (through cigarette smoking) during the 1st and 2nd months of pregnancy compared with the mothers of heterosexual women (Ellis & Cole-Harding, 2001). Prenatal stress levels or drug agents may alter levels of intrauterine hormones to which the fetus is exposed, consequently influencing central nervous system development. Prenatal stress levels are also known to disrupt PPI in rats (Lehman, Stohr, & Feldon, 2000). Nonetheless, it should be noted that the association between maternal reports of prenatal stress and homosexuality have not been consistently reported (Bailey, Willerman, & Parks, 1991; Schmidt & Clement, 1995). Moreover, there were no differences in the number of smokers compared with nonsmokers between men and women, or between homosexuals and heterosexuals, in our sample ($p < .10$).

An additional possibility is that the differences seen in PPI between heterosexual and homosexual women are reflective of a “third variable” linking PPI and sexual orientation. One reviewer of this article suggested that reduced PPI may be associated with elevated scores on certain personality measures on which homosexual women may also score higher. Of note here is that homosexual women typically score in male-typical directions on masculinity–femininity and gender-diagnosticity measures (the former being a traditional measure of psychological gender, whereas the latter uses occupational and activity interests to index gender role orientation; Lippa, 2000). Alternatively, the often reported (retrospectively and prospectively) higher rates of childhood gender nonconformity in homosexual men’s and women’s sexual orientation may also constitute an “endophenotype” for differences in PPI rather than sexual orientation per se (Bailey & Zucker, 1995).

Given that PPI reflects sensorimotor gating, our observations show that sexual orientation influences information processing at a fundamental cognitive level. The startle response itself is reflexive, but its inhibition by a prepulse (i.e., PPI) is not. PPI is thought to reflect reduced processing of incoming information while processing of the initial stimulus (prepulse) is still ongoing. This has led to the suggestion that PPI is an information processing mechanism reflecting sensorimotor gating that protects the individual from sensory overload (Graham, 1975). This conceptualization—that impaired inhibitory processes underlying deficient PPI lead to cognitive fragmentation—is supported by a number of empirical observations in schizophrenia. Impaired PPI predicts poor responses on the Ego Impairment Index (Human Experience subscale), a measure of thought disorder (Perry & Braff 1994; Perry, Geyer & Braff, 1999) and correlates positively with a number of other cognitive deficits, for example, poor performance on the Wisconsin Card Sort Test (Butler et al. 1991) and distractibility (Karper et al., 1996) in schizophrenic patients. Thus, although not suggesting that homosexuality is associated with psychopathology or impaired cognitive functioning, differences in basic information processing may account, at least in part, for previously reported sexual orientation-related differences in some higher order cognitive and motor functions (Hall & Kimura, 1995; Wegesin, 1998b). For example, homosexual women show male-typical visuomotor functions (e.g., targeted throwing of objects) compared with heterosexual women (Hall & Kimura, 1995).

The lack of difference between heterosexual and homosexual men is not unprecedented. In a number of measures, such as the ratio of the second to fourth finger lengths, OAEs, and AEPS (McFadden & Champlin, 2000), homosexual men show male-typical or even hypermasculine patterns, perhaps indicative of greater androgenization. Although contrary to the notion that homosexual men have brains that are globally female-typical, recent theories posit a nonlinear influence of masculinizing factors, particularly prenatal androgens, on male sexual orientation, such that...
both low or high levels of these factors cause shifts from male-typical behavior (Rahman & Wilson, 2003b; McFadden, 2002).

It should be emphasized that the differences reported here are for groups and do not mean that PPI can be used as a reliable indicator of sexual orientation in individuals. However, if the sexual orientation effects reported here are found to be robust and reliable in future work, then the PPI model has implications for, among other areas, gender and sexual orientation-related differences in psychiatric disorders, according to which lesbians and gay men tend to show cross-sex shifts in certain sexually dimorphic mental illnesses, such as depression (Bailey, 1999; Sandfort, de Graaf, Bijl, & Schnabel, 2001). The present findings suggest that PPI deserves serious consideration as a noninvasive measure of individual differences in sensorimotor functions.

References


Graham, F. K. (1975). The more or less startling effects of weak prestimuli. Psychophysiology, 12, 238–248.


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